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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 498/22, C07H 19/044, A61K 31/55, 31/70 // (C07D 498/22, 311:00, 273:00, 209:00, 209:00, 209:00)

A1

(11) International Publication Number: WO 95/32976

(43) International Publication Date: 7 December 1995 (07.12.95)

(21) International Application Number: PCT/EP95/01911

(22) International Filing Date: 19 May 1995 (19.05.95)

(30) Priority Data:
1715/94-4 1 June 1994 (01.06.94) CH

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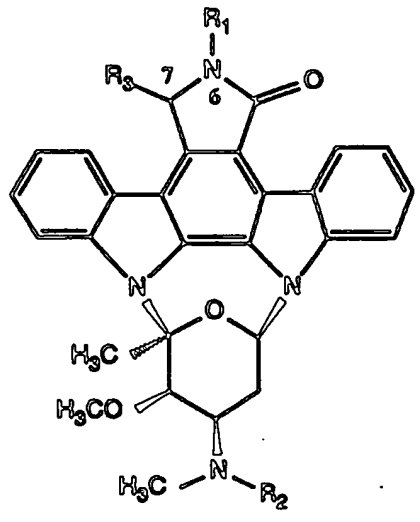
(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

(54) Title: POLYCYCLIC LACTAM DERIVATIVES FOR SENSITIZING MULTIDRUG-RESISTANT CELLS TO ANTITUMOUR AGENTS

(57) Abstract

Staurosporin derivatives of formula (I), wherein R_1 is formyl, an aliphatic hydrocarbon radical having up to 29 carbon atoms that is unsubstituted or substituted by aryl, or is an aryl radical, R_2 is an aliphatic, carbocyclic, carbocyclic-aliphatic, heterocyclic or heterocyclic-aliphatic radical each having up to 29 carbon atoms that is other than C_1-C_5alkyl, or is a heterocyclic or heterocyclic-aliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms, or is an acyl radical having up to 30 carbon atoms that is other than benzoyl, benzyloxycarbonyl, lower alkanoyl or α-aminoacyl having a free or protected amino group, and R_3 is hydrogen, hydroxy, lower alkoxy or oxo, are described. These derivatives can be used for avoiding or removing multidrug resistance to antitumour agents, such as vinblastine or adriamycin.



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POLYCYCLIC LACTAM DERIVATIVES FOR SENSITZING MULTIDRUG-RESISTANT CELLS TO ANTITUMOUR AGENTS

The invention relates to staurosporin derivatives, to a process for the preparation thereof, to pharmaceutical compositions comprising those compounds, to the use thereof as medicaments and to processes for the preparation of the intermediates.

Staurosporin, which forms the basis of the derivatives according to the invention, was isolated as early as 1977 from cultures of Streptomyces staurosporeus AWAYA, TAKAHASHI and OMURA, sp. nov. AM 2282, see S. Omura *et al.*, J. Antibiot. 30, 275-281 (1977). Hitherto, only the relative, but not the absolute, configuration of staurosporin was known. The absolute configuration has been published only recently by N. Funato *et al.*, Tetrahedron Letters 35:8, 1251-1254 (1994) and corresponds to the mirror image of the structure previously used in the literature to indicate the relative configuration of staurosporin. Accordingly, the Tetrahedron Letters publication recommends verbatim "that the stereochemical notation for staurosporine which has been in common use hitherto should be revised". Although the absolute configuration was not known hitherto, it was clearly established by the term "staurosporin derivative". In this text, the new formulae are used.

Staurosporin and most of the staurosporin derivatives known hitherto show a strong inhibitory action on protein kinase C. Protein kinase C, which is dependent upon phospholipids and calcium, occurs within the cell in several forms and participates in various fundamental processes, such as signal transmission, proliferation and differentiation and also secretion of hormones and neurotransmitters. Activation of that enzyme is brought about either by receptor-mediated hydrolysis of phospholipids of the cell membrane or by direct interaction with certain tumour-promoting active agents. The sensitivity of the cell towards receptor-mediated signal transmission can be significantly influenced by modifying the activity of protein kinase C (as the signal transmitter). Compounds that are capable of influencing the activity of protein kinase C may be used as tumour-inhibiting, anti-inflammatory, immuno-modulating and antibacterial active ingredients and may even be of interest as agents against atherosclerosis and disorders of the cardiovascular system and the central nervous system.

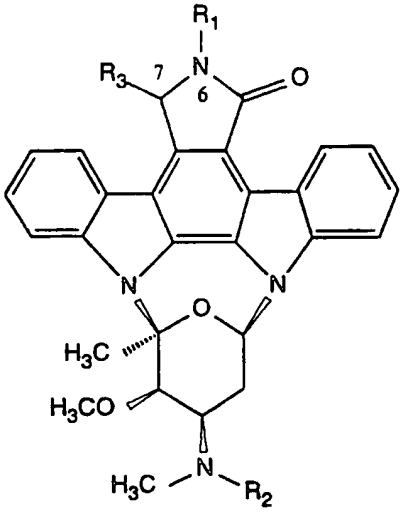
The inhibitory action on protein kinase C is weakened by a factor of from approximately 20 to over 1000 if the lactam nitrogen of staurosporin carries instead of hydrogen another substituent, that is to say, if the substituent R_1 in formula I shown hereinafter is other than hydrogen. Especially when in formula I below the radical R_2 is, at the same time, also

other than hydrogen, the inhibitory action on protein kinase C is to all practical purposes lost. When the substituent R_1 in formula I shown below is other than hydrogen, the anti-tumour activity also decreases markedly. It is presumably for that reason that only few staurosporin derivatives wherein R_1 is other than hydrogen are described in the literature, although much work has been undertaken in the field in recent years and very many derivatives wherein R_1 is hydrogen have been prepared. Thus, the compound corresponding to formula I below wherein R_1 is benzyl, R_2 is benzoyl and R_3 is hydrogen, has mostly been mentioned only as a negative control.

The appearance of resistance to classical cytostatic agents is a great problem in cancer chemotherapy. The resistance is in many cases accompanied by a reduction in the intracellular concentration of active ingredient. That reduction is often associated with the appearance of a membrane-bound 170 kilodalton glycoprotein (Pgp). That protein acts as a pump having a broad specificity and is capable of transporting frequently used anti-tumour agents, such as the Vinca alkaloids, anthracyclins, podophyllotoxins and actinomycin D, out of the cell.

Surprisingly, it has now been found that staurosporin derivatives of formula I shown hereinbelow are capable of fully re-sensitising multidrug-resistant cells to the action of anti-tumour agents, such as cytostatics, as can be demonstrated *inter alia* by the example of resistant human KB-8511 cells. This is achieved even though, as mentioned above, all derivatives show a greatly weakened inhibitory action or no inhibitory action at all on protein kinase C and the anti-tumour activity is also markedly reduced. Also surprising is the high degree of sensitisation. In that respect, the staurosporin derivatives of formula I are roughly equivalent to the analogous derivatives wherein R_1 is hydrogen. Compared with a combination of a conventional cytostatic agent and a staurosporin derivative having pronounced anti-tumour activity and inhibitory action on protein kinase C, a combination of a conventional cytostatic agent with a staurosporin derivative of formula I shown hereinbelow has the advantage that the side-effects associated with the protein kinase C inhibitory action do not occur or occur only in a very much weaker form. The administration of protein kinase C inhibiting staurosporin derivatives results, for example in dogs, in nausea to the point of vomiting. The latter is understandably disadvantageous, especially for an orally administered anti-tumour agent, since active ingredient may also be vomited, with the result that the dose of active ingredient effectively taken may be different from the intended and administered dose.

The invention relates to staurosporin derivatives of formula I



(I)

wherein

R_1 is formyl, an aliphatic hydrocarbon radical having up to 29 carbon atoms that is unsubstituted or substituted by aryl, or is an aryl radical,

R_2 is an aliphatic, carbocyclic, carbocyclic-aliphatic, heterocyclic or heterocyclic-aliphatic radical each having up to 29 carbon atoms that is other than C_1-C_5alkyl, or is a heterocyclic or heterocyclic-aliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms, or is an acyl radical having up to 30 carbon atoms that is other than benzoyl, benzyloxycarbonyl, lower alkanoyl or α-aminoacyl having a free or protected amino group, and

R_3 is hydrogen, hydroxy, lower alkoxy or oxo,

and salts of such compounds of formula I having at least one salt-forming group.

Unless stated otherwise, in this disclosure, organic radicals referred to as "lower" contain not more than 7, preferably not more than 4, carbon atoms.

An unsubstituted aliphatic hydrocarbon radical R_1 having up to 29 carbon atoms is an acyclic hydrocarbon radical having up to 29 carbon atoms, especially up to 18, and preferably up to 12, carbon atoms, and is saturated or unsaturated. Unsaturated radicals are those containing one or more, especially conjugated and/or isolated, multiple bonds (double and/or triple bonds). An unsubstituted aliphatic hydrocarbon radical is especially a linear or branched lower alkyl, lower alkenyl, lower alkadienyl or lower alkynyl radical.

Lower alkyl is, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl, or also n-pentyl, isopentyl, n-hexyl, isohexyl or n-heptyl; lower alkenyl is, for example, allyl, propenyl, isopropenyl, 2- or 3-methallyl or 2- or 3-butenyl; lower alkadienyl is, for example, 1-penta-2,4-dienyl; lower alkynyl is, for example, propargyl or 2-butynyl. In corresponding unsaturated radicals, the double bond is located especially in a position higher than the α-position with respect to the free valency.

An aliphatic hydrocarbon radical R_1 substituted by aryl is one in which an aliphatic hydrocarbon radical, especially one having a maximum of 7, preferably a maximum of 4, carbon atoms, such as especially methyl, ethyl and vinyl, carries one or more aryl radicals as defined hereinbelow.

An aryl radical is especially a phenyl radical, but also a naphthyl radical, such as 1- or 2-naphthyl, a biphenylyl radical, such as especially 4-biphenylyl, or also an anthryl, fluorenyl or azulenyl radical, or an aromatic analogue thereof having one or more saturated rings. Preferred aryl-lower alkyl and aryl-lower alkenyl radicals are, for example, phenyl-lower alkyl or phenyl-lower alkenyl with a terminal phenyl radical, for example benzyl, phenethyl, 1-, 2- or 3-phenylpropyl, diphenylmethyl (benzhydryl), trityl and cinnamyl, and also 1- or 2-naphthylmethyl. Of the aryl radicals that carry acyclic radicals, such as lower alkyl, there are to be mentioned, in particular, o-, m- and p-tolyl and xylyl radicals having methyl radicals situated in different positions.

An aliphatic radical R_2 having up to 29 carbon atoms that is other than C_1-C_5alkyl is an unsubstituted C_6-C_{29}alkyl radical, such as especially C_7-C_{29}alkyl, preferably C_{10}-C_{22}alkyl, most especially C_{10}-C_{18}alkyl, or is a C_1-C_{29}alkyl radical substituted by acyclic substituents, especially such a substituted C_1-C_7alkyl radical, or a linear or branched lower alkenyl, lower alkadienyl or lower alkynyl radical that is unsubstituted or substituted by acyclic substituents.

A carbocyclic radical R_2 is especially a mono-, bi- or poly-cyclic cycloalkyl, cycloalkenyl or cycloalkadienyl radical, or a corresponding aryl radical. Preference is given to radicals having a maximum of 14, especially 12, ring carbon atoms and 3- to 8-, preferably 5- to 7- and especially 6-membered rings, it also being possible for them to carry one or more, for example two, acyclic radicals, especially lower alkyl radicals, or further carbocyclic radicals. Cycloalkyl has especially from 3 up to and including 10 carbon atoms and is, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl,

and also bicyclo[2,2,2]octyl, 2-bicyclo[2,2,1]heptyl and adamantyl, each of which may also be substituted by 1, 2 or more, for example lower, alkyl radicals, especially methyl radicals; cycloalkenyl is, for example, one of the monocyclic cycloalkyl radicals already mentioned that has a double bond in the 1-, 2- or 3-position.

An aryl radical is, for example, one of those mentioned above.

Carbocyclic-aliphatic radicals R_2 are those in which an aliphatic radical, especially one having a maximum of 7, preferably a maximum of 4, carbon atoms, such as especially methyl, ethyl and vinyl, carries one or more carbocyclic, if desired aromatic, radicals as defined above. There may be mentioned, in particular, cycloalkyl-lower alkyl and aryl-lower alkyl radicals, and the analogues thereof that are unsaturated in the ring and/or chain and that carry the ring at the terminal carbon atom of the chain. Cycloalkyl-lower alkyl or -lower alkenyl is, for example, methyl, 1- or 2-ethyl, 1- or 2-vinyl, 1-, 2- or 3-propyl or allyl substituted by one of the above-mentioned cycloalkyl radicals, those substituted at the end of the linear chain being preferred.

Heterocyclic radicals R_2 are especially monocyclic, but also bi- or poly-cyclic, aza-, thia-, oxa-, thiaza-, oxaza-, diaza-, triaza- or tetraza-cyclic radicals of aromatic character, and corresponding partially saturated or, especially, completely saturated heterocyclic radicals of that kind, it being possible for such radicals, where appropriate, for example like the above-mentioned carbocyclic or aryl radicals, to carry further acyclic, carbocyclic or heterocyclic radicals and/or to be mono-, di- or poly-substituted by functional groups. Such radicals are, especially, unsubstituted or substituted monocyclic radicals having one nitrogen, oxygen or sulfur atom, such as 2-aziridinyl, and especially aromatic radicals of that kind, such as pyrryl, for example 2-pyrryl or 3-pyrryl, pyridyl, for example 2-, 3- or 4-pyridyl, or also thienyl, for example 2- or 3-thienyl, or furyl, for example 2-furyl; analogous bicyclic radicals having one nitrogen, oxygen or sulfur atom are, for example, indolyl, such as 2- or 3-indolyl, quinolyl, such as 2- or 4-quinolyl, isoquinolyl, such as 3- or 5-isoquinolyl, benzofuranyl, such as 2-benzofuranyl, chromenyl, such as 3-chromenyl, or benzothienyl, such as 2- or 3-benzothienyl; preferred monocyclic and bicyclic radicals having a plurality of hetero atoms are, for example, imidazolyl, such as 2-imidazolyl, pyrimidinyl, such as 2- or 4-pyrimidinyl, oxazolyl, such as 2-oxazolyl, isoxazolyl, such as 3-isoxazolyl, or thiazolyl, such as 2-thiazolyl, and benzimidazolyl, such as 2-benzimidazolyl, benzoxazolyl, such as 2-benzoxazolyl, or quinazolyl, such as 2-quinazolinyl. Corresponding partially saturated or, especially, completely saturated

analogous radicals also come into consideration, such as 2-tetrahydrofuryl, 4-tetrahydrofuryl, 2- or 3-pyrrolidyl, 2-, 3- or 4-piperidyl, and also 2- or 3-morpholinyl, 2- or 3-thiomorpholinyl, 2-piperazinyl and N,N'-bis-lower alkyl-2-piperazinyl radicals.

Heterocyclic-aliphatic (heterocyclic-acyclic) radicals R_2 are derived especially from acyclic radicals having a maximum of 7, preferably a maximum of 4, carbon atoms, for example from those mentioned above, and may carry one, two or more heterocyclic radicals, for example those mentioned above, it also being possible for the ring to be bonded to the chain by one of its nitrogen atoms. The acyclic moiety in heterocyclic-acyclic radicals has, for example, the meanings given for the corresponding carbocyclic-aliphatic (carbocyclic-acyclic) radicals.

If substituted, the above-mentioned radicals may be substituted by one, two or more substituents (functional groups) of the same kind or of different kinds; the following substituents come into consideration especially: free, etherified and esterified hydroxy groups; mercapto, lower alkylthio and unsubstituted or substituted phenylthio groups; halogen atoms, such as chlorine and fluorine, but also bromine and iodine; oxo groups that are in the form of formyl (i.e. aldehydo) and keto groups and in the form of corresponding acetals and ketals, respectively; azido and nitro groups; primary, secondary and, preferably, tertiary amino groups, primary and secondary amino groups that are protected by conventional protecting groups, acylamino groups and diacylamino groups, and free or functionally modified sulfo groups, such as sulfamoyl groups or sulfo groups in salt form. None of these functional groups should be located at the carbon atom from which the free valency extends and they are all preferably separated therefrom by 2 or even more carbon atoms. Further possible substituents are free and functionally modified carboxy groups, such as carboxy groups in salt form or esterified carboxy groups, or carbamoyl, ureido or guanidino groups optionally carrying one or two hydrocarbon radicals, and cyano groups.

An etherified hydroxy group present as a substituent is, for example, a lower alkoxy group, such as a methoxy, ethoxy, propoxy, isopropoxy, butoxy or tert-butoxy group, which may also be substituted. For example, such a lower alkoxy group may be substituted, for example mono-, di- or poly-substituted, by halogen atoms, especially in the 2-position, such as in the 2,2,2-trichloroethoxy, 2-chloroethoxy and 2-iodoethoxy radical, or substituted, preferably mono-substituted, by hydroxy or by lower alkoxy radicals, especially in the 2-position, such as in the 2-methoxyethoxy radical. An especially preferred form of the etherified hydroxy groups exists in oxa-alkyl radicals, in which

one or more of the carbon atoms in a preferably linear alkyl have been replaced by oxygen atoms that are preferably separated from one another by a plurality of (especially 2) carbon atoms, so that they form an optionally repeatedly recurring $(-O-CH_2-CH_2)_n-$ group wherein n = 1 to 14. Such etherified hydroxy groups are also unsubstituted or substituted phenoxy radicals and phenyl-lower alkoxy radicals, such as especially benzyloxy, benzhydryloxy and triphenylmethoxy (trityloxy), and heterocyclyloxy radicals, such as especially 2-tetrahydropyranyloxy. The groupings methylenedioxy and ethylenedioxy may be regarded as special etherified hydroxy groups; the former as a rule bridges 2 adjacent carbon atoms, especially in aryl radicals, and the latter is bonded to one and the same carbon atom and may be regarded as a protecting group for oxo.

The expression "etherified hydroxy groups" is also to be understood in this context as including silylated hydroxy groups, as occur, for example, in tri-lower alkylsilyloxy, such as trimethylsilyloxy and dimethyl-tert-butylsilyloxy, or phenyl-di-lower alkylsilyloxy or lower alkyl-diphenylsilyloxy.

An esterified hydroxy group present as a substituent is, for example, lower alkanoyloxy.

An esterified carboxy group present as a substituent is one in which the hydrogen atom has been replaced by one of the hydrocarbon radicals characterised above, preferably a lower alkyl or phenyl-lower alkyl radical; there may be mentioned as examples of an esterified carboxy group lower alkoxycarbonyl or phenyl-lower alkoxycarbonyl that is unsubstituted or substituted in the phenyl moiety, especially the methoxy-, ethoxy-, tert-butoxy- or benzyloxy-carbonyl group, and also a lactonised carboxy group.

A primary amino group $-NH_2$ present as a substituent may also be in protected form. A secondary amino group carries, in place of one of the two hydrogen atoms, a hydrocarbyl radical, preferably an unsubstituted hydrocarbyl radical, such as one of those mentioned above, especially lower alkyl, and may also be in protected form.

A tertiary amino group occurring as a substituent carries 2 different or, preferably, identical hydrocarbyl radicals (including the heterocyclic radicals), such as the unsubstituted hydrocarbyl radicals characterised above, especially lower alkyl.

A preferred amino group is one of the formula $R_4(R_5)N-$, wherein R_4 and R_5 are each independently of the other hydrogen, unsubstituted acyclic C_1-C_7hydrocarbyl (such as,

especially, a C_1-C_4alkyl or C_2-C_4alkenyl) or monocyclic aryl, aralkyl or aralkenyl that has a maximum of 10 carbon atoms and that is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen and/or by nitro, it being possible for the carbon-containing radicals to be bonded to one another by a carbon-carbon bond or by an oxygen atom, by a sulfur atom, or by a nitrogen atom that is unsubstituted or substituted by hydrocarbyl. In such a case, they form together with the nitrogen atom of the amino group a nitrogen-containing heterocyclic ring. The following may be mentioned as examples of especially preferred free amino groups: di-lower alkylamino, such as dimethylamino, diethylamino, pyrrolidino, piperidino, morpholino, thiomorpholino and piperazino or 4-methylpiperazino, or diphenylamino and dibenzylamino each unsubstituted or substituted, especially in the phenyl moiety, for example by lower alkyl, lower alkoxy, halogen and/or by nitro; and, of the protected amino groups, especially lower alkoxycarbonylamino, such as tert-butoxycarbonylamino, phenyl-lower alkoxycarbonylamino, such as 4-methoxybenzyloxycarbonylamino, and 9-fluorenylmethoxycarbonylamino.

Preferred substituents are C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, also carboxy, C_1-C_4alkoxycarbonyl, methylenedioxy and/or cyano.

An acyl radical R_2 having up to 30 carbon atoms that is other than benzoyl, benzyloxycarbonyl, lower alkanoyl or α-aminoacyl having a free or protected amino group is derived from such an optionally functionally modified carboxylic acid, an organic sulfonic acid or a free or esterified phosphoric acid, such as pyro- or ortho-phosphoric acid.

An acyl derived from an optionally functionally modified carboxylic acid, which is designated Ac^1, is especially one of the partial formula Z-C(=W)- wherein W is oxygen, sulfur or imino, and Z is hydrocarbyl R^o_a, hydrocarbyloxy R^o-O- or an amino group, especially one of the formula $R_4(R_5)N$-.

The hydrocarbyl (hydrocarbon radical) R^o is an acyclic (aliphatic) hydrocarbon radical, a carbocyclic hydrocarbon radical or a carbocyclic-acyclic hydrocarbon radical other than unsubstituted benzyl, each radical having up to 29 carbon atoms, especially up to 18, and preferably up to 12, carbon atoms, and is saturated or unsaturated and unsubstituted or substituted. It may also contain in place of one, two or more carbon atoms identical or different hetero atoms, such as especially oxygen, sulfur and nitrogen, in the acyclic and/or cyclic moiety; in the latter case, it is referred to as a heterocyclic radical (heterocyclyl radical) or a heterocyclic-acyclic radical.

The hydrocarbyl (hydrocarbon radical) R^o_a is benzyl or a hydrocarbyl R^o that is other than unsubstituted C_1-C_7alkyl, other than the decarboxy radical of an α-amino acid having a free amino group or an amino group protected by an amino-protecting group and other than unsubstituted phenyl.

Unsaturated radicals are those which contain one or more, especially conjugated and/or isolated, multiple bonds (double and/or triple bonds). The term "cyclic radicals" also includes aromatic radicals, for example those wherein at least one 6-membered carbocyclic or one 5- to 8-membered heterocyclic ring contains the maximum number of non-cumulated double bonds. Carbocyclic radicals wherein at least one ring is in the form of a 6-membered aromatic ring (i.e. a benzene ring) are referred to as aryl radicals.

An acyclic unsubstituted hydrocarbon radical is especially a linear or branched lower alkyl, lower alkenyl, lower alkadienyl or lower alkynyl radical. Lower alkyl is, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl, or also n-pentyl, isopentyl, n-hexyl, isohexyl or n-heptyl; lower alkenyl is, for example, allyl, propenyl, isopropenyl, 2- or 3-methallyl or 2- or 3-butenyl; lower alkadienyl is, for example, 1-penta-2,4-dienyl; lower alkynyl is, for example, propargyl or 2-butynyl. In corresponding unsaturated radicals, the double bond is located especially in a position higher than the α-position with respect to the free valency.

A carbocyclic hydrocarbon radical is especially a mono-, bi- or poly-cyclic cycloalkyl, cycloalkenyl or cycloalkadienyl radical, or a corresponding aryl radical. Preference is given to radicals having a maximum of 14, especially 12, ring carbon atoms and 3- to 8-, preferably 5- to 7- and especially 6-membered rings, it also being possible for them to carry one or more, for example two, acyclic radicals, for example those mentioned above, and especially the lower alkyl radicals, or further carbocyclic radicals. Carbocyclic-acyclic radicals are those in which an acyclic radical, especially one having a maximum of 7, preferably a maximum of 4, carbon atoms, such as especially methyl, ethyl and vinyl, carries one or more carbocyclic, if desired aromatic, radicals as defined above, with the exception of benzyl. There may be mentioned, in particular, cycloalkyl-lower alkyl and aryl-lower alkyl radicals, and the analogues thereof that are unsaturated in the ring and/or chain and that carry the ring at the terminal carbon atom of the chain.

Cycloalkyl radicals, aryl radicals, heterocyclic radicals and heterocyclic-acyclic (hetero-

cyclic-aliphatic) radicals are, for example, those mentioned above.

As has already been mentioned, a hydrocarbyl (including a heterocyclyl) may be substituted by one, two or more substituents (functional groups) of the same kind or of different kinds. The substituents mentioned above especially come into consideration.

Preferred compounds of formula I according to the invention are, for example, those wherein hydrocarbyl R^o has the following preferred meanings of an acyclic hydrocarbyl: a C_1-C_{20}alkyl, a C_2-C_{20}hydroxyalkyl the hydroxy group of which is located in any position apart from the 1-position, preferably in the 2-position, a cyano-[C_1-C_{20}]alkyl the cyano group of which is preferably located in the 1- or ω-position, or a carboxy-[C_1-C_{20}]alkyl the carboxy group of which is preferably located in the 1- or ω-position and may, where appropriate, also be in salt form or in the form of a C_1-C_4alkyl ester (C_1-C_4alkoxycarbonyl) or a benzyl ester (benzyloxycarbonyl), and a C_3-C_{20}alkenyl the free valency of which is not located at the same carbon atom as is the double bond, all of the mentioned radicals, with the exception of those with the C_3-C_5alkyl basic structure, having a linear (unbranched) alkyl chain; also a linear (mono-, di- to hexa)-oxaalkyl having from 4 to 20 chain members, wherein one or more of the carbon atoms, from C-3 onward, of a linear C_4-C_{20}alkyl has been replaced by oxygen atoms that are separated from one another by at least 2 carbon atoms and are preferably located in positions 3, 6, 9, 12, 15 and 18.

Preferred compounds of formula I according to the invention are also those wherein hydrocarbyl R^o has the following preferred meanings of a carbocyclic or heterocyclic, or also carbocyclic-acyclic or heterocyclic-acyclic, hydrocarbyl: a bicyclic or, preferably, monocyclic aryl, especially phenyl, or also naphthyl, that may carry one or more of the following substituents: halogen atoms, especially fluorine, chlorine and bromine, C_1-C_4-alkyl radicals, especially methyl, C_1-C_4alkoxy groups, especially methoxy, methylenedioxy, nitro groups and/or carboxy groups that may be free, in salt form or in the form of C_1-C_4alkyl esters, especially methoxycarbonyl or ethoxycarbonyl. Preferably, the aryl radicals carry not more than 2 substituents, especially those of the same kind, or carry only a single substituent; most especially, they are unsubstituted. A preferred heterocyclic hydrocarbyl (hydrocyclyl) is, for example, one that is analogous to the aryl radicals given prominence above and that contains, instead of one or two carbon atoms, in each case a hetero atom, especially nitrogen, such as a pyridyl or quinolyl, or quinazolyl, respectively, wherein the free valency is located at a carbon atom and accordingly can also be substituted. Preferred carbocyclic-acyclic and heterocyclic-acyclic hydrocarbyl radicals are

those wherein two or three, but preferably only one, of the above-defined cyclic radicals, preferably the unsubstituted cyclic radicals, is carried by a C_1-C_3alkyl, all of them preferably being located at one carbon atom, preferably the terminal carbon atom; but with the exception of unsubstituted benzyl.

Especially preferred compounds of formula I are those wherein R^o is C_1-C_7alkyl, especially C_1-C_4alkyl, hydroxy-C_2-C_{18}alkyl, especially hydroxy-C_2-C_{14}alkyl, cyano-C_1-C_7alkyl, especially cyano-C_1-C_4alkyl, carboxy-C_1-C_7alkyl, especially carboxy-C_1-C_4alkyl, C_1-C_7-alkoxy-carbonyl-C_1-C_7alkyl, especially C_1-C_4alkoxy-carbonyl-C_1-C_4alkyl, benzyloxy-carbonyl-C_1-C_7alkyl, especially benzyloxycarbonyl-C_1-C_4alkyl, C_3-C_7alkenyl, phenyl, naphthyl, pyridyl, quinolyl, or quinazolyl, or phenyl-C_1-C_7alkyl, especially phenyl-C_1-C_3-alkyl, it also being possible for the respective aromatic radicals to be substituted by C_1-C_7-alkyl, especially C_1-C_4alkyl, C_1-C_7alkoxy, especially C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, also carboxy, C_1-C_4alkoxy-carbonyl, methylenedioxy and/or by cyano, the hydroxy group in a correspondingly substituted alkyl radical being located especially in the 2-position, and the cyano, carboxy, alkoxy-carbonyl, benzyloxy-carbonyl or phenyl group in a correspondingly substituted alkyl radical being located especially in the 1- or ω-position.

Especially preferred compounds of formula I are those wherein R^o is C_1-C_4alkyl, such as methyl or ethyl, hydroxy-C_2-C_{14}alkyl, such as 2-hydroxy-propyl, -hexyl, -decyl or -tetradecyl, cyano-C_1-C_4alkyl, such as 2-cyanoethyl, carboxy-C_1-C_4alkyl, such as carboxymethyl, C_1-C_4alkoxycarbonyl-C_1-C_4alkyl, such as methoxycarbonyl-methyl or -ethyl, C_3-C_7alkenyl, such as allyl, or phenyl, the hydroxy group in a correspondingly substituted alkyl preferably being located in the 2-position and the cyano, carboxy or alkoxycarbonyl group being located especially in the 1- or ω-position.

An acyl derived from an organic sulfonic acid, which is designated Ac^2, is especially one of the partial formula R^o_c-SO_2- wherein R^o_c is benzyl or a hydrocarbyl R^o having the general meanings given above and the meanings given prominence above, the latter meanings generally representing also in this case the preferred selection.

An acyl derived from a free or esterified phosphoric acid, which is designated Ac^3, is especially one of the partial formula $R_4O(R_5O)P(=O)$-, wherein R_4 and R_5 each independently have the general meanings given above and the meanings given prominence above. R_4 and R_5 preferably have the same meaning.

Preferred acyl radicals Ac^1 are acyl radicals of a carboxylic acid that are characterised by the partial formula R^o_a-CO- wherein R^o_a has one of the above-mentioned general and preferred meanings of the hydrocarbyl radical R^o_a, and that are accordingly derived from a corresponding, unsubstituted or substituted acyclic, carbocyclic, carbocyclic-acyclic, heterocyclic or heterocyclic-acyclic monocarboxylic acid. A preferred hydrocarbyl in such an acyl is, for example, an unsubstituted C_8-C_{19}alkyl, especially a C_{11}-C_{19}- or C_{13}-C_{19}-alkyl, especially one that has a linear chain, or a C_1-C_{19}alkyl, especially a C_1-C_7alkyl that carries the following substituents: a carboxy group that may also be in salt form or in the form of a cyano group or a C_1-C_4alkyl ester (C_1-C_4alkoxycarbonyl group) and that is preferably located in the ω-position, or one or more halogen atoms, especially fluorine or chlorine, which are preferably located vicinal to the carbonyl group. Another preferred acyl is a bicyclic or, especially, monocyclic aroyl that is other than unsubstituted benzoyl and that may carry one or more of the following substituents: halogen atoms, especially chlorine or fluorine, nitro groups, C_1-C_4alkyl radicals, especially methyl, hydroxy groups and etherified hydroxy groups, especially C_1-C_4alkoxy, such as methoxy, phenoxy and methylenedioxy, and carboxy groups that may also be in salt form or in the form of a cyano group or a C_1-C_4alkyl ester (C_1-C_4alkoxycarbonyl). Preferably, the aroyl radicals carry not more than 2, and especially only one, such substituent. Also preferred are analogous heteroaroyl radicals, especially those derived from pyridine, furan, thiophene and imidazole and from analogues thereof having a fused benzo ring (such as quinoline, isoquinoline, benzofuran and benzimidazole) and that are also unsubstituted or substituted as indicated above. Preferred acyl radicals of that kind are also derived from monocyclic aryl-alkenyl, for example corresponding aryl-C_2-C_5alkenyl, such as benzyl and styryl (i.e. phenacetyl and cinnamoyl) and can also be substituted in the manner given above. There may be mentioned by way of example C_{2-30}acyl radicals R_2 that are derived from the following carboxylic acids: aliphatic monocarboxylic acids having from 8 to 20 carbon atoms, such as lauric, myristic, palmitic and stearic acid, and oleic acid, elaidic acid, linoleic acid and linolenic acid; halogenated lower alkanecarboxylic acids, such as chloroacetic acid, trifluoro- or trichloro-acetic acid, bromoacetic or α-bromoisovaleric acid; carbocyclic or carbocyclic-acyclic monocarboxylic acids, for example cyclo-propane-, cyclopentane- and cyclohexane-carboxylic acid or cyclopentane- or cyclo-hexane-acetic acid or -propionic acid, respectively; aromatic carbocyclic carboxylic acids, with the exception of benzoic acid, that may be mono- or poly-substituted as indicated above; aryl- or aryloxy-lower alkanecarboxylic acids and analogues thereof that are unsaturated in the chain, for example phenylacetic or phenoxyacetic acids that are

unsubstituted or substituted as indicated above, phenylpropionic acids and cinnamic acids; and heterocyclic acids, for example furan-2-carboxylic acid, 5-tert-butylfuran-2-carboxylic acid, thiophene-2-carboxylic acid, nicotinic or isonicotinic acid, 4-pyridinepropionic acid, and pyrrole-2- or -3-carboxylic acids that are unsubstituted or substituted by lower alkyl radicals; also dicarboxylic acids, such as oxalic acid, malonic acid, mono- or di-lower alkylmalonic acids, succinic acid, glutaric acid, adipic acid, erucic acid, maleic acid, or a phthalic, quinolinic, isoquinolinic or phenylsuccinic acid that is unsubstituted or substituted by halogen, such as fluorine, chlorine or bromine, and/or by lower alkyl, hydroxy, lower alkoxy and by nitro, and also glutamic acids and aspartic acid, the latter two acids preferably having protected amino groups. In this case, the second carboxy group may not only be free but may also be functionally modified, for example in the form of a C_1-C_4alkyl ester or in the form of a salt, preferably in the form of a physiologically tolerable salt, with a salt-forming basic component. There come into consideration especially metal or ammonium salts, such as alkali metal and alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, or ammonium salts with ammonia or suitable organic amines.

Another preferred acyl Ac^1 is derived from monoesters of carbonic acid and is characterised by the partial formula R^o-O-CO-. Among especially preferred hydrocarbyl radicals R^o in these derivatives there are to be mentioned, for example, the following: acyclic hydrocarbyl, especially a C_1-C_{20}alkyl, preferably a linear C_1-C_{20}alkyl, that may be substituted by a carboxy group, preferably in a functionally modified form, such as a salt, cyano or a C_1-C_4alkyl ester, that is preferably located in the ω-position, or an analogous linear (mono- to hexa-)oxaalkyl having from 4 to 20 chain members, especially one characterised above as being especially preferred. Also preferred within this definition of R^o are substituted phenyl radicals, for example those mentioned above as being preferred.

Yet another preferred acyl Ac^1 is derived from amides of carbonic acid (or also thiocarbonic acid) and is characterised by the formula $R_4(R_5)N$-C(=W)- wherein R_4 and R_5 are as defined above and W is sulfur or especially oxygen.

The acyl radical Ac^2 is derived from an acyclic, carbocyclic or heterocyclic, or also a carbocyclic-acyclic or heterocyclic-acyclic sulfonic acid and corresponds to the mentioned partial formula R^o-SO_2-. Of the compounds according to the invention that carry the radical Ac^2 special prominence is to be given to those wherein R^o is a C_1-C_7alkyl or, especially, bicyclic or especially a monocyclic aryl, such as especially phenyl, that may be

substituted in a manner analogous to that described above for the aroyl radicals given prominence. Prominence is also to be given to bicyclic and monocyclic aromatic heterocyclyl radicals of analogous structure, in which one or two of the carbon atoms have been replaced by hetero atoms, such as pyrimidyl, for example 2- or 4-pyrimidyl, quinolyl or isoquinolyl. The heterocyclyl radicals also may carry substituents, especially those given prominence for aroyl (in that case, for example, a hydroxy derivative is, by virtue of tautomeric shifting of the double bond, the same as a dihydro-oxo derivative).

The acyl radical Ac^3 derived from a phosphoric acid is, for example, an acyl radical that is derived from pyrophosphoric acid or, especially, from orthophosphoric acid and that may also be in a functionally modified form, for example in the form of a salt, a hydrocarbyl ester or an amide. Of the compounds of formula I according to the invention wherein R_1 is Ac^3 prominence is to be given especially to those wherein Ac^3 corresponds to the partial formula $R_4O(R_5O)P(=O)$- wherein R_4 and R_5 have the general meanings given above and the meanings given special prominence above and are preferably identical and are hydrogen or an unsubstituted C_1-C_7alkyl, especially a linear C_1-C_7alkyl, such as especially methyl or ethyl, or alternatively a phenyl that is unsubstituted or substituted, especially by C_1-C_4alkyl, C_1-C_4alkoxy, halogens and/or by nitro.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula Z-C(=W)- wherein W is oxygen, also sulfur, and Z is C_1-C_7alkyl that is substituted by halogen, carboxy or by C_1-C_4alkoxy-carbonyl.

Especially preferred are those compounds of formula I wherein R_2 is an acyl of the partial formula Z-C(=W)- wherein W is oxygen or also sulfur, and Z is pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuryl or benzimidazolyl, each of which is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4alkoxy-carbonyl, methylenedioxy and/or by cyano.

Especially preferred are those compounds of formula I wherein R_2 is an acyl of the partial formula R^o-CO- wherein R^o is phenyl that is substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, such as fluorine or chlorine, nitro, trifluoromethyl, carboxy or by C_1-C_4alkoxy-carbonyl.

Especially preferred are those compounds of formula I wherein R_2 is an acyl of the partial

formula R^o-SO_2- wherein R^o is C_1-C_7alkyl, especially C_1-C_4alkyl.

Especially preferred are those compounds of formula I wherein R_2 is an acyl of the partial formula R^o_c-SO_2- wherein R^o_c is phenyl, or also pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl, each of which is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4-alkoxy-carbonyl, methylenedioxy and/or by cyano.

Especially preferred are those compounds of formula I wherein R_2 is an acyl of the partial formula R^o_c-SO_2- wherein R^o_c is phenyl or C_1-C_4alkyl- or halo-substituted phenyl or isoquinolyl, such as 5-isoquinolyl.

Especially preferred are those compounds of formula I wherein R_2 is an acyl of the partial formula R^o-O-CO- wherein R^o is C_1-C_7alkyl, especially C_1-C_4alkyl.

Especially preferred are those compounds of formula I wherein R_2 is an acyl of the partial formula R^o-O-CO- wherein R^o is pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl, each of which is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4alkoxy-carbonyl, methylenedioxy and/or by cyano.

Especially preferred are those compounds of formula I wherein R_2 is an acyl of the partial formula $R_4(R_5)N$-C(=W)- wherein W is sulfur or, especially, oxygen, R_4 is hydrogen and R_5 is C_1-C_7alkyl, especially C_1-C_4alkyl, C_3-C_7alkenyl or phenyl, or also pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl, each of which is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4alkoxy-carbonyl, methylenedioxy and/or by cyano.

Lower alkoxy R_3 is preferably methoxy.

Depending on their nature, the compounds according to the invention may, provided they contain salt-forming groups, also be in the form of salts, especially pharmaceutically acceptable, i.e. physiologically tolerable, salts. For isolation or purification purposes it is also possible to use pharmaceutically unacceptable salts. Only pharmaceutically acceptable salts are used therapeutically and these are preferred.

Thus, compounds of formula I having free acid groups, such as a free sulfo, phosphoryl or carboxy group, may be in the form of a salt, preferably a physiologically tolerable salt, with a salt-forming basic component. There come into consideration especially metal or ammonium salts, such as alkali metal and alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, or ammonium salts with ammonia or suitable organic amines, especially tertiary monoamines and heterocyclic bases, for example tri-ethylamine, tri-(2-hydroxyethyl)-amine, N-ethylpiperidine or N,N'-dimethylpiperazine.

Compounds according to the invention of basic character may also be in the form of addition salts, especially in the form of acid addition salts with inorganic and organic acids, but also in the form of quaternary salts. Thus, for example, compounds of formula I that carry a basic group, such as an amino group, as a substituent may form acid addition salts with commonly used acids. Suitable acids are, for example, hydrohalic acids, for example hydrochloric and hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid or perchloric acid, and aliphatic, alicyclic, aromatic or heterocyclic carboxylic or sulfonic acids, such as formic, acetic, propionic, succinic, glycolic, lactic, malic, tartaric, citric, fumaric, maleic, hydroxymaleic, oxalic, pyruvic, phenylacetic, benzoic, p-aminobenzoic, anthranilic, p-hydroxybenzoic, salicylic, p-aminosalicylic, embonic, methanesulfonic, ethanesulfonic, hydroxyethanesulfonic, ethylenedisulfonic, halobenzenesulfonic, toluene-sulfonic, naphthalenesulfonic acids or sulfanilic acid, also methionine, tryptophan, lysine or arginine, or also ascorbic acid.

The staurosporin derivatives of formula I are capable of fully re-sensitising multidrug-resistant cells to the action of anti-tumour agents, such as cytostatics, as is demonstrated in the Examples section of this text in the case of resistant human KB-8511 cells. Such anti-tumour agents are, for example, doxorubicin, daunorubicin, vincristine, etoposide, taxol, mitomycin C, actinomycin D, mitozantrone and, especially, vinblastine and adriamycin. The staurosporin derivatives of formula I and pharmaceutically acceptable salts of such derivatives having at least one salt-forming group can therefore be used in combination with one of those anti-tumour agents for the treatment of tumour diseases.

As mentioned above, the inhibitory action of the compounds of formula I on protein kinase C virtually no longer exists or, compared with the analogous compounds wherein R_1 is hydrogen, is greatly weakened. To determine the protein kinase C inhibitory action, pig brain protein kinase C is used, which is purified in accordance with the procedure described by T. Uchida and C.R. Filburn in J. Biol. Chem. 259, 12311-4 (1984). The

protein kinase C inhibitory action of the compounds of formula I was formerly determined according to the methodology of D. Fabbro *et al.*, Arch. Biochem. Biophys. 239, 102-111 (1985). The pig brain protein kinase C used according to the methodology mentioned is a mixture of different subtypes (isotypes) of protein kinase C. For that reason, nowadays, pure, recombinant isotypes are mostly used instead of pig brain protein kinase C.

Recombinant PKC isotypes are cloned, expressed and purified as follows:

The preparation of various proteins with the aid of baculoviruses and their cloning and isolation from Sf9 insect cells is carried out as described by M.D. Summers and G.E. Smith, "A manual method for baculovirus vectors and insect cell culture procedure", Texas Agricul. Exptl. Station Bull. (1987), 1555. The construction and isolation of recombinant viruses for the expression of PKC-α (bovine), PKC-β1 (human), PKC-β2 (human) and PKC-γ (human/bovine hybrid) in Sf9 cells is carried out as described by Stabel *et al.*, [S. Stabel, M. Liyanage and D. Frith, "Expression of protein kinase C isozymes in insect cells and isolation of recombinant proteins", Meth. Neurosc. (1993)]. The preparation of the PKC isotypes in Sf9 cells is carried out as specified by Stabel *et al.* (see above), and the purification of the enzymes is performed by the method described in the publication by McGlynn *et al.* [E. McGlynn, J. Liebetanz, S. Reutener, J. Wood, N.B. Lydon, H. Hofstetter, M. Vanek, T. Meyer and D. Fabbro, "Expression and partial characterization of rat protein kinase C-δ and protein kinase C-ζ in insect cells using recombinant baculovirus", J. Cell. Biochem. 49, 239-250 (1992)]. For the generation of recombinant PKC-δ (rat), PKC-ε (rat), PKC-ζ (rat) and PKC-η (mouse) and the expression and purification thereof the procedure described by Liyanage *et al.* ["Protein kinase C group B members PKC-δ, -ε, -ζ and PKC-λ: Comparison of properties of recombinant proteins in vitro and in vivo", Biochem. J. 283, 781-787 (1992)] and McGlynn *et al.* (see above) is followed, with the addition that, for the expression of PKC-η, the transfer vector pAc360 is used [V. Luckow and M.D. Summers, "Trends in the development of baculovirus expression", Biotechnology 6, 47-55 (1988)].

Measurement of the activity of the recombinant PKC isotypes obtained by the above method is carried out in the absence of lipid and calcium (co-factors). Protamine sulfate, which is phosphorylated in the absence of co-factors, is used as a substrate for this. The activity of the enzymes reflects the transfer of ^{32}P from γ-[^{32}P]-ATP to protamine sulfate. Protamine sulfate is a mixture of polypeptides that each comprise four C-terminal arginine residues. Measurement of the phosphate incorporation is carried out under the following

conditions: 100 µl of the reaction mixture contain in final concentrations 20 mmol TRIS-HCl pH 7.4, 10 mmol $Mg[NO_3]_2$, 0.5 mg/ml protamine sulfate, 10 µmol ATP (0.1 µCi γ-[^{32}P]-ATP; 10 Ci/mol; Amersham, Little Chalfont, United Kingdom), various concentrations of inhibitory substances and 0.5-2.5 U (Units; one unit is the enzyme quantity that transfers one nanomol of ^{32}P from the above-mentioned γ-[^{32}P]-ATP to Histon H1 [Sigma, type V-S] in one minute per milligram of protein) of the enzymes. The reaction is initiated by adding the enzymes and transferring to 32°C. The reaction time is 20 minutes. Thereafter, the reaction is stopped by dropping aliquots of 50 µl onto P81 chromatography paper (Whatman, Maidstone, United Kingdom). After removing unbound γ-[^{32}P]-ATP and nucleotide fractions by washing procedures as described by J.J. Witt and R. Roskoski, "Rapid protein kinase assay using phospho-cellulose-paper absorption", Anal. Biochem. 66, 253-258 (1975), the phosphorylation of the substrate is determined by scintillation measurement. In this test, the compounds of formula I generally do not inhibit the various isotypes of protein kinase C (PKC) until they are at a concentration IC_{50} that is greater by a factor of from about 20 to over 1000 than the IC_{50} values that are found for analogous compounds wherein R_1 is hydrogen.

Preferred are compounds of formula I that have virtually no significant inhibitory action on protein kinase C.

Also preferred are compounds of formula I wherein R_1 is other than unsubstituted lower alkyl.

Very preferred are compounds of formula I wherein R_1 is lower alkyl, such as especially methyl, or benzyl, R_2 is lower alkoxycarbonyl, such as especially ethoxycarbonyl or tert-butoxycarbonyl, tetrahydropyran-4-yloxy-lower alkanoyl, such as especially 2-tetrahydro-pyran-4-yloxy-acetyl or (D)-O-tetrahydropyran-4-yl-lactoyl, or is lower alkyl substituted by lower alkoxycarbonyl, such as especially methoxycarbonyl, or by carboxy, and R_3 is hydroxy, lower alkoxy or preferably hydrogen or oxo, and salts of such compounds having at least one salt-forming group.

Very preferred are especially also compounds of formula I wherein R_1 is lower alkyl, such as especially methyl, or benzyl, R_2 is tetrahydropyran-4-yloxy-lower alkanoyl, such as especially 2-tetrahydropyran-4-yloxy-acetyl or (D)-O-tetrahydropyran-4-yl-lactoyl, or is lower alkyl substituted by lower alkoxycarbonyl, such as especially methoxycarbonyl, or by carboxy, and R_3 is hydroxy, lower alkoxy or preferably hydrogen or oxo, and salts of

such compounds having at least one salt-forming group.

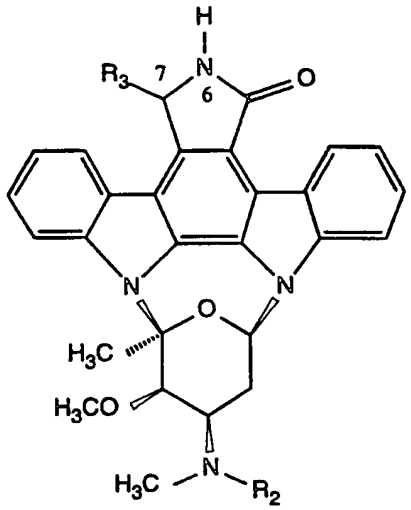
Very preferred are especially compounds of formula I wherein R_1 is benzyl, R_2 is lower alkoxycarbonyl, such as especially ethoxycarbonyl or tert-butoxycarbonyl, tetrahydropyran-4-yloxy-lower alkanoyl, such as especially 2-tetrahydropyran-4-yloxy-acetyl or (D)-O-tetrahydropyran-4-yl-lactoyl, or is lower alkyl substituted by lower alkoxycarbonyl, such as especially methoxycarbonyl, or by carboxy, and R_3 is hydroxy, lower alkoxy or preferably hydrogen or oxo, and salts of such compounds having at least one salt-forming group.

Especially preferred are the compounds of formula I described in the Examples.

Most preferred are the compounds of formula I described in the Examples, with the exception of N-(tert-butoxycarbonyl)-6-methyl-staurosporin.

The compounds of formula I and salts of such compounds having at least one salt-forming group are prepared by processes known *per se*. The process according to the invention comprises

a) reacting a compound of formula II



(II),

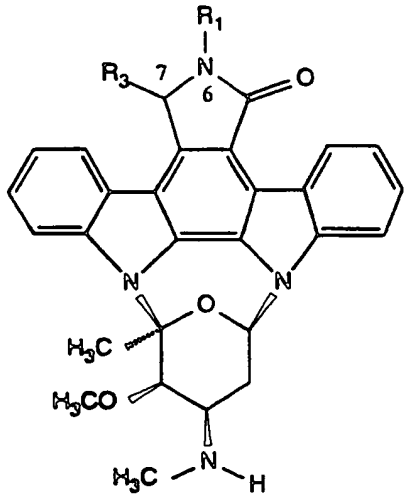
wherein the substituents are as defined above, any functional groups present therein being, if necessary, in protected form, or a salt of such a compound having at least one salt-

forming group, with a compound of formula

$$R_1Y \qquad (III),$$

wherein R_1 is as defined above, any functional groups present therein being, if necessary, in protected form, and Y is a leaving group or an additional single bond the other end of which replaces a hydrogen atom in the radical R_1, or with a salt of such a compound having at least one salt-forming group, and removing any protecting groups, or

b) reacting a compound of formula IV



(IV),

wherein the substituents are as defined above, any functional groups present therein being, if necessary, in protected form, or a salt of such a compound having at least one salt-forming group, with a compound of formula

$$R_2X \qquad (V),$$

wherein R_2 is as defined above, any functional groups present in the radical R_2 being, if necessary, in protected form, and X is a leaving group or an additional single bond the other end of which replaces a hydrogen atom in the radical R_2, or with a salt of such a compound having at least one salt-forming group, and removing any protecting groups, and, if desired, converting a resulting compound of formula I into a different compound of formula I and/or converting a compound of formula I obtained in free form into a salt

thereof and/or converting a compound of formula I obtained in the form of a salt into its free form or into a different salt.

The way in which the above-mentioned process variants are carried out is explained in detail below:

General remarks:
The end products of formula I may contain substituents that can also be used as protecting groups in starting materials for the preparation of other end products of formula I. Within the scope of this text, therefore, unless otherwise apparent from the context, the term "protecting group" denotes only a readily removable group that is not a component part of the particular desired end product of formula I.

Process a): Free functional groups that may be present in compounds of formulae II and III, which are preferably protected by readily removable protecting groups, are especially free amino or carboxy groups. It may also be advantageous to protect free hydroxy. Functional groups that are intended to participate in the desired reaction are not, of course, protected.

Protecting groups and the methods by which they are introduced and removed are described, for example, in "Protective Groups in Organic Chemistry", Plenum Press, London, New York 1973, and in "Methoden der organischen Chemie", Houben-Weyl, 4th edition, Vol. 15/1, Georg-Thieme-Verlag, Stuttgart 1974 and also in Theodora W. Greene, "Protective Groups in Organic Synthesis", John Wiley & Sons, New York 1981. It is characteristic of protecting groups that they can be removed easily, i.e. without undesirable secondary reactions taking place, for example by solvolysis, reduction, photolysis or also under physiological conditions.

A protected amino group may, for example, be in the form of a readily cleavable acylamino, arylmethylamino, etherified mercaptoamino, 2-acyl-lower alk-1-en-yl-amino, silylamino or stannylamino group or in the form of an azido group.

In a corresponding acylamino group, acyl is, for example, the acyl radical of an organic carboxylic acid having, for example, up to 18 carbon atoms, especially of an unsubstituted or substituted, for example halo- or aryl-substituted, alkanecarboxylic acid or an unsubstituted or substituted, for example halo-, lower alkoxy- or nitro-substituted, benzoic acid, or

of a carbonic acid semiester. Such acyl groups are, for example, lower alkanoyl, such as formyl, acetyl or propionyl, halo-lower alkanoyl, such as 2-haloacetyl, especially 2-chloro-, 2-bromo-, 2-iodo-, 2,2,2-trifluoro- or 2,2,2-trichloro-acetyl, unsubstituted or substituted, for example halo-, lower alkoxy- or nitro-substituted, benzoyl, for example benzoyl, 4-chlorobenzoyl, 4-methoxybenzoyl or 4-nitrobenzoyl, or lower alkoxycarbonyl that is branched in the 1-position of the lower alkyl radical or suitably substituted in the 1- or 2-position, especially tert-lower alkoxycarbonyl, for example tert-butoxycarbonyl, aryl-methoxycarbonyl having one or two aryl radicals which are preferably phenyl that is unsubstituted or is mono- or poly-substituted, for example, by lower alkyl, especially tert-lower alkyl, such as tert-butyl, lower alkoxy, such as methoxy, hydroxy, halogen, for example chlorine, and/or by nitro, such as unsubstituted or substituted benzyloxycarbonyl, for example 4-nitrobenzyloxycarbonyl, or substituted diphenylmethoxycarbonyl, for example benzhydryloxycarbonyl or di-(4-methoxyphenyl)-methoxycarbonyl, aroyl-methoxycarbonyl wherein the aroyl group is preferably benzoyl that is unsubstituted or substituted, for example, by halogen, such as bromine, for example phenacyloxycarbonyl, 2-halo-lower alkoxycarbonyl, for example 2,2,2-trichloroethoxycarbonyl, 2-bromoethoxy-carbonyl or 2-iodoethoxycarbonyl, or 2-(trisubstituted silyl)-ethoxycarbonyl wherein each substituent independently is an aliphatic, araliphatic, cycloaliphatic or aromatic hydro-carbon radical having up to 15 carbon atoms that is unsubstituted or substituted, for example, by lower alkyl, lower alkoxy, aryl, halogen or by nitro, such as corresponding, unsubstituted or substituted, lower alkyl, phenyl-lower alkyl, cycloalkyl or phenyl, for example 2-tri-lower alkylsilylethoxycarbonyl, such as 2-trimethylsilylethoxycarbonyl or 2-(di-n-butyl-methyl-silyl)-ethoxycarbonyl, or 2-triarylsilylethoxycarbonyl, such as 2-tri-phenylsilylethoxycarbonyl.

Other acyl radicals that are suitable as amino-protecting groups are also corresponding radicals of organic phosphoric, phosphonic or phosphinic acids, such as di-lower alkyl-phosphoryl, for example dimethylphosphoryl, diethylphosphoryl, di-n-propylphosphoryl or diisopropylphosphoryl, dicycloalkylphosphoryl, for example dicyclohexylphosphoryl, unsubstituted or substituted diphenylphosphoryl, for example diphenylphosphoryl, unsub-stituted or substituted, for example nitro-substituted, di-(phenyl-lower alkyl)-phosphoryl, for example dibenzylphosphoryl or di-(4-nitrobenzyl)-phosphoryl, unsubstituted or substi-tuted phenyloxy-phenyl-phosphonyl, for example phenyloxyphenyl-phosphonyl, di-lower alkylphosphinyl, for example diethylphosphinyl, or unsubstituted or substituted diphenyl-phosphinyl, for example diphenylphosphinyl.

In an arylmethylamino group that is a mono-, di- or, especially, a tri-arylmethylamino group, the aryl radicals are, especially, unsubstituted or substituted phenyl radicals. Such groups are, for example, benzyl-, diphenylmethyl- and, especially, trityl-amino.

An etherified mercapto group in an amino group protected by such a radical is especially arylthio or aryl-lower alkylthio wherein aryl is especially phenyl that is unsubstituted or substituted, for example, by lower alkyl, such as methyl or tert-butyl, lower alkoxy, such as methoxy, halogen, such as chlorine, and/or by nitro. A corresponding amino-protecting group is, for example, 4-nitrophenylthio.

In a 2-acyl-lower alk-1-en-1-yl radical that can be used as an amino-protecting group, acyl is, for example, the corresponding radical of a lower alkanecarboxylic acid, of a benzoic acid that is unsubstituted or substituted, for example, by lower alkyl, such as methyl or tert-butyl, lower alkoxy, such as methoxy, halogen, such as chlorine, and/or by nitro, or especially of a carbonic acid semiester, such as a carbonic acid lower alkyl semiester. Corresponding protecting groups are especially 1-lower alkanoyl-prop-1-en-2-yl, for example 1-acetyl-prop-1-en-2-yl, or 1-lower alkoxycarbonyl-prop-1-en-2-yl, for example 1-ethoxycarbonyl-prop-1-en-2-yl.

Preferred amino-protecting groups are acyl radicals of carbonic acid semiesters, especially tert-butoxycarbonyl, benzyloxycarbonyl that is unsubstituted or substituted, for example as indicated, for example 4-nitro-benzyloxycarbonyl, or diphenylmethoxycarbonyl, or 2-halo-lower alkoxycarbonyl, such as 2,2,2-trichloroethoxycarbonyl, also trityl or formyl. Carboxy groups are usually protected in esterified form, such ester groupings being readily cleavable under mild conditions. Carboxy groups protected in that manner contain as esterifying groups especially lower alkyl groups that are branched in the 1-position or suitably substituted in the 1- or 2-position. Preferred carboxy groups in esterified form are *inter alia* tert-lower alkoxycarbonyl, for example tert-butoxycarbonyl, arylmethoxy-carbonyl having one or two aryl radicals which are phenyl radicals that are unsubstituted or mono- or poly-substituted, for example, by lower alkyl, such as tert-lower alkyl, for example tert-butyl, lower alkoxy, such as methoxy, hydroxy, halogen, for example chlorine, and/or by nitro, such as benzyloxycarbonyl that is unsubstituted or substituted, for example as mentioned above, for example 4-methoxybenzyloxycarbonyl or 4-nitro-benzyloxycarbonyl, or diphenylmethoxycarbonyl that is unsubstituted or substituted, for example as mentioned above, for example diphenylmethoxycarbonyl or di-(4-methoxy-phenyl)-methoxycarbonyl, 1-lower alkoxy-lower alkoxycarbonyl, such as methoxy-

methoxycarbonyl, 1-methoxyethoxycarbonyl or 1-ethoxymethoxycarbonyl, 1-lower alkylthio-lower alkoxycarbonyl, such as 1-methylthiomethoxycarbonyl or 1-ethylthioethoxycarbonyl, aroylmethoxycarbonyl wherein the aroyl group is benzoyl that is unsubstituted or substituted, for example by halogen, such as bromine, for example phenacyloxycarbonyl, 2-halo-lower alkoxycarbonyl, for example 2,2,2-trichloroethoxycarbonyl, 2-bromoethoxycarbonyl or 2-iodoethoxycarbonyl, or 2-(trisubstituted silyl)ethoxycarbonyl wherein each substituent independently is an aliphatic, araliphatic, cycloaliphatic or aromatic hydrocarbon radical that is unsubstituted or substituted, for example, by lower alkyl, lower alkoxy, aryl, halogen and/or by nitro, such as corresponding, unsubstituted or substituted, lower alkyl, phenyl-lower alkyl, cycloalkyl or phenyl, for example 2-tri-lower alkylsilylethoxycarbonyl, 2-trimethylsilylethoxycarbonyl or 2-(di-n-butyl-methyl-silyl)-ethoxycarbonyl, or 2-triarylsilylethoxycarbonyl, such as 2-triphenylsilylethoxycarbonyl.

The organic silyl and stannyl radicals mentioned above and hereinafter contain preferably lower alkyl, especially methyl, as substituents of the silicon or tin atoms. Corresponding silyl or stannyl groups are especially tri-lower alkylsilyl, especially trimethylsilyl, or dimethyl-tert-butyl-silyl, or correspondingly substituted stannyl, for example tri-n-butylstannyl.

Preferred protected carboxy groups are tert-lower alkoxycarbonyl, such as tert-butoxycarbonyl, and especially benzyloxycarbonyl that is unsubstituted or substituted, for example, as mentioned above, such as 4-nitrobenzyloxycarbonyl, or diphenylmethoxycarbonyl, especially 2-(trimethylsilyl)ethoxycarbonyl.

Hydroxy-protecting groups are, for example, acyl radicals, such as unsubstituted or substituted, for example halo-substituted, lower alkanoyl, such as 2,2-dichloroacetyl, or acyl radicals of carbonic acid semiesters, especially tert-butoxycarbonyl, unsubstituted or substituted benzyloxycarbonyl, for example 4-nitrobenzyloxycarbonyl, or diphenylmethoxycarbonyl, or 2-halo-lower alkoxycarbonyl, such as 2,2,2-trichloroethoxycarbonyl, also trityl or formyl, or organic silyl or stannyl radicals, or readily removable etherifying groups, such as tert-lower alkyl, for example tert-butyl, 2-oxa- or 2-thia-aliphatic or -cycloaliphatic hydrocarbon radicals, especially 1-lower alkoxy-lower alkyl or 1-lower alkylthio-lower alkyl, for example methoxymethyl, 1-methoxy-ethyl, 1-ethoxy-ethyl, methylthiomethyl, 1-methylthioethyl or 1-ethylthioethyl, or 2-oxa- or 2-thia-cycloalkyl having 5 or 6 ring atoms, for example tetrahydrofuryl or 2-tetrahydropyranyl or corresponding thia analogues, and also unsubstituted or substituted 1-phenyl-lower alkyl, such

as unsubstituted or substituted benzyl or diphenylmethyl, suitable substituents of the phenyl radicals being, for example, halogen, such as chlorine, lower alkoxy, such as methoxy, and/or nitro.

The removal of protecting groups that are not constituents of the desired end product of formula I, for example the carboxy-, amino-, hydroxy- or carbamoyl-protecting groups, is effected in a manner known *per se*, for example by means of solvolysis, especially hydrolysis, alcoholysis or acidolysis, or by means of reduction, especially hydrogenolysis or chemical reduction, as appropriate stepwise or simultaneously, it being possible also to use enzymatic methods, for example acidolysis, such as treatment with trifluoroacetic acid or formic acid, or reduction, such as treatment with zinc and acetic acid, or with hydrogen and a hydrogenation catalyst, such as a palladium-on-carbon catalyst.

When several protected functional groups are present, the protecting groups are preferably so chosen that more than one such group can be removed simultaneously.

A protected amino group is freed in a manner known *per se* and, according to the nature of the protecting groups, in various ways, preferably by solvolysis or reduction. 2-Halo-lower alkoxycarbonylamino (where appropriate after conversion of a 2-bromo-lower alkoxycarbonylamino group into a 2-iodo-lower alkoxycarbonylamino group), aroylmethoxycarbonylamino or 4-nitrobenzyloxycarbonylamino can be cleaved, for example, by treatment with a suitable chemical reducing agent, such as zinc in the presence of a suitable carboxylic acid, such as aqueous acetic acid. Aroylmethoxycarbonylamino can be cleaved also by treatment with a nucleophilic, preferably salt-forming, reagent, such as sodium thiophenolate, and 4-nitrobenzyloxycarbonylamino also by treatment with an alkali metal dithionite, for example sodium dithionite. Unsubstituted or substituted diphenylmethoxycarbonylamino, tert-lower alkoxycarbonylamino or 2-(tri-substituted silyl)-ethoxycarbonylamino, can be cleaved by treatment with a suitable acid, for example formic acid or trifluoroacetic acid, or with hydrochloric acid in ethyl acetate or dioxane; unsubstituted or substituted benzyloxycarbonylamino can be cleaved, for example, by means of hydrogenolysis, i.e. by treatment with hydrogen in the presence of a suitable hydrogenation catalyst, such as a palladium catalyst; unsubstituted or substituted triarylmethylamino or formylamino can be cleaved, for example, by treatment with an acid, such as a mineral acid, for example hydrochloric acid, or an organic acid, for example formic, acetic or trifluoroacetic acid, where appropriate in the presence of water; and an amino group protected by an organic silyl group can be freed, for example, by means of hydro-

lysis or alcoholysis. An amino group protected by 2-haloacetyl, for example 2-chloroacetyl, can be freed by treatment with thiourea in the presence of a base, or with a thiolate salt, such as an alkali metal thiolate, of thiourea, and subsequent solvolysis, such as alcoholysis or hydrolysis, of the resulting condensation product. An amino group protected by 2-substituted silylethoxycarbonyl can be converted into the free amino group also by treatment with a salt of hydrofluoric acid that yields fluoride anions.

Tert-lower alkoxycarbonyl, lower alkoxycarbonyl substituted in the 2-position by an organic silyl group or in the 1-position by lower alkoxy or by lower alkylthio, or unsubstituted or substituted diphenylmethoxycarbonyl can be converted into free carboxy, for example, by treatment with a suitable acid, such as formic acid or trifluoroacetic acid, where appropriate with the addition of a nucleophilic compound, such as phenol or anisole. Unsubstituted or substituted benzyloxycarbonyl can be freed, for example, by means of hydrogenolysis, i.e. by treatment with hydrogen in the presence of a metal hydrogenation catalyst, such as a palladium catalyst. In addition, suitably substituted benzyloxycarbonyl, such as 4-nitrobenzyloxycarbonyl, can be converted into free carboxy also by chemical reduction, for example by treatment with an alkali metal dithionite, such as sodium dithionite, or with a reducing metal, for example zinc, or a reducing metal salt, such as a chromium(II) salt, for example chromium(II) chloride, customarily in the presence of a hydrogen-yielding agent that, together with the metal, is capable of producing nascent hydrogen, such as an acid, especially a suitable carboxylic acid, such as an unsubstituted or substituted, for example hydroxy-substituted, lower alkanecarboxylic acid, for example acetic acid, formic acid, glycolic acid, diphenylglycolic acid, lactic acid, mandelic acid, 4-chloromandelic acid or tartaric acid, or in the presence of an alcohol or thiol, water preferably being added. By treatment with a reducing metal or metal salt, as described above, 2-halo-lower alkoxycarbonyl (where appropriate after conversion of a 2-bromo-lower alkoxycarbonyl group into a corresponding 2-iodo-lower alkoxycarbonyl group) or aroylmethoxycarbonyl can also be converted into free carboxy. Aroylmethoxycarbonyl can be cleaved also by treatment with a nucleophilic, preferably salt-forming, reagent, such as sodium thiophenolate or sodium iodide. Substituted 2-silylethoxycarbonyl can also be converted into free carboxy by treatment with a salt of hydrofluoric acid that yields the fluoride anion, such as an alkali metal fluoride, for example sodium or potassium fluoride, in the presence of a macrocyclic polyether ("crown ether"), or with a fluoride of an organic quaternary base, such as tetra-lower alkylammonium fluoride or tri-lower alkyl-arylammonium fluoride, for example tetraethylammonium fluoride or tetrabutylammonium fluoride, in the presence of an aprotic, polar solvent, such as dimethyl

sulfoxide or N,N-dimethylacetamide.

A hydroxy group protected by a suitable acyl group, an organic silyl group or by unsubstituted or substituted 1-phenyl-lower alkyl is freed analogously to a correspondingly protected amino group. Hydroxy protected by unsubstituted or substituted 1-phenyl-lower alkyl, for example benzyl, is freed preferably by catalytic hydrogenation, for example in the presence of a palladium-on-carbon catalyst. A hydroxy group protected by 2,2-dichloroacetyl is freed, for example, by basic hydrolysis, and a hydroxy group etherified by tert-lower alkyl or by a 2-oxa- or 2-thia-aliphatic or -cycloaliphatic hydrocarbon radical is freed by acidolysis, for example by treatment with a mineral acid or a strong carboxylic acid, for example trifluoroacetic acid. Hydroxy etherified by an organic silyl radical, for example trimethylsilyl, can also be freed with a salt of hydrofluoric acid that yields fluoride anions, for example tetrabutylammonium fluoride.

If Y is a leaving group that is bonded to a non-aromatic carbon atom in the radical R_1, Y is especially a reactive esterified hydroxy group, i.e. one that is esterified by a strong inorganic acid, such as a hydrohalic acid (for example hydrochloric, hydrobromic or hydriodic acid), by an oxygen-containing mineral acid, such as phosphoric acid and, especially, sulfuric acid, or by a strong organic, such as aliphatic or aromatic, sulfonic acid (for example methane- and ethane- or benzene-, p-toluene-, p-nitrobenzene- and p-chlorobenzene-sulfonic acid).

If Y is a leaving group that is bonded to an aromatic carbon atom in the radical R_1, for example to a phenyl radical, Y is especially a diazonium group.

If Y is an additional single bond the other end of which replaces a hydrogen atom in the radical R_1, R_1Y is, for example, an alkene, especially one in which the double bond has been additionally activated by a structural peculiarity, as in 2-methylpropene, or by substitution, such as especially in acrylonitrile. Also included in the definition of Y is a single bond the other end of which is not bonded directly to a carbon atom in the radical R_1 but is bonded to a hetero atom occurring as a substituent, such as oxygen (for example in a hydroxy group) or nitrogen (in an amino group) (replacing a hydrogen atom of that group). Especially preferred reagents of that kind contain the α-epoxide (oxirane) or α-imine (aziridine) grouping and serve as an advantageous source of radicals R^o having a 2-hydroxyalkyl grouping or 2-aminoalkyl grouping, respectively.

If R_1 is formyl, the reagent R_1Y is a reactive carboxylic acid derivative. Y therein is, for example, a reactive esterified hydroxy group, such as especially halogen. Such reactive carboxylic acid derivatives of formula III are especially reactive activated esters or reactive anhydrides, or also reactive cyclic amides, it also being possible for the activation of the carboxylic acid of formula R_1-OH used as acylating agent to be performed *in situ* in the presence of the compound of formula II.

Activated esters of acids are especially esters that are unsaturated at the linking carbon atom of the esterifying radical, for example of the vinyl ester type, such as vinyl esters proper (obtainable, for example, by transesterification of a corresponding ester with vinyl acetate; activated vinyl ester method), carbamoylvinyl esters (obtainable, for example, by treatment of the corresponding acid with an isoxazolium reagent; 1,2-oxazolium or Woodward method), or 1-lower alkoxyvinyl esters (obtainable, for example, by treatment of the corresponding acid with a lower alkoxyacetylene; ethoxyacetylene method), or esters of the amidino type, such as N,N'-disubstituted amidino esters (obtainable, for example, by treatment of the corresponding acid with a suitable N,N'-disubstituted carbodiimide, for example N,N'-dicyclohexylcarbodiimide; carbodiimide method), or N,N-disubstituted amidino esters (obtainable, for example, by treatment of the corresponding acid with an N,N-disubstituted cyanamide; cyanamide method), suitable aryl esters, especially phenyl esters suitably substituted by electron-attracting substituents (obtainable, for example, by treatment of the corresponding acid with a suitably substituted phenol, for example 4-nitrophenol, 4-methylsulfonylphenol, 2,4,5-trichlorophenol, 2,3,4,5,6-pentachlorophenol or 4-phenyldiazophenol, in the presence of a condensation agent, such as N,N'-dicyclohexylcarbodiimide; activated aryl esters method), cyanomethyl esters (obtainable, for example, by treatment of the corresponding acid with chloroacetonitrile in the presence of a base; cyanomethyl esters method), thioesters, especially unsubstituted or substituted, for example nitro-substituted, phenylthio esters (obtainable, for example, by treatment of the corresponding acid with unsubstituted or substituted, for example nitro-substituted, thiophenols, *inter alia* by the anhydride or carbodiimide method; activated thiol esters method), amino or amido esters (obtainable, for example, by treatment of the corresponding acid with an N-hydroxyamino or N-hydroxyamido compound, for example N-hydroxysuccinimide, N-hydroxypiperidine, N-hydroxyphthalimide or 1-hydroxybenzotriazole, for example by the anhydride or carbodiimide method; activated N-hydroxy esters method), or silyl esters (which are obtainable, for example, by treatment of the corresponding acid with a silylating agent, for example hexamethyldisilazane, and which readily react with hydroxy groups but not with amino groups).

Anhydrides of acids may be symmetric or preferably mixed anhydrides of those acids, for example anhydrides with inorganic acids, such as acid halides, especially acid chlorides (obtainable, for example, by treatment of the corresponding acid with thionyl chloride, phosphorus pentachloride or oxalyl chloride; acid chloride method), azides (obtainable, for example, from a corresponding acid ester *via* the corresponding hydrazide and treatment thereof with nitrous acid; azide method), anhydrides with carbonic acid semiderivatives, such as corresponding esters, for example carbonic acid lower alkyl semiesters (obtainable, for example, by treatment of the corresponding acid with haloformic, such as chloroformic, acid lower alkyl esters or with a 1-lower alkoxycarbonyl-2-lower alkoxy-1,2-dihydroquinoline, for example 1-lower alkoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; mixed O-alkylcarbonic acid anhydrides method), or anhydrides with dihalogenated, especially dichlorinated, phosphoric acid (obtainable, for example, by treatment of the corresponding acid with phosphorus oxychloride; phosphorus oxychloride method), or anhydrides with organic acids, such as mixed anhydrides with organic carboxylic acids (obtainable, for example, by treatment of the corresponding acid with an unsubstituted or substituted lower alkane- or phenylalkane-carboxylic acid halide, for example phenylacetic acid chloride, pivalic acid chloride or trifluoroacetic acid chloride; mixed carboxylic acid anhydrides method) or with organic sulfonic acids (obtainable, for example, by treatment of a salt, such as an alkali metal salt, of the corresponding acid with a suitable organic sulfonic acid halide, such as a lower alkane- or aryl-, for example methane- or p-toluene-sulfonic acid chloride; mixed sulfonic acid anhydrides method) and symmetric anhydrides (obtainable, for example, by condensation of the corresponding acid in the presence of a carbodiimide or 1-diethylaminopropyne; symmetric anhydrides method).

Suitable cyclic amides are especially amides having five-membered diazacycles of aromatic character, such as amides with imidazoles, for example imidazole (obtainable, for example, by treatment of the corresponding acid with N,N'-carbonyldiimidazole; imidazolide method), or pyrazoles, for example 3,5-dimethylpyrazole (obtainable, for example, *via* the acid hydrazide by treatment with acetylacetone; pyrazolide method).

As mentioned, derivatives of acids used as acylating agents can also be formed *in situ*. Thus, for example, N,N'-disubstituted amidino esters can be formed *in situ* by reacting a mixture of the starting material of formula II and the acid used as acylating agent in the presence of a suitable N,N-disubstituted carbodiimide, for example N,N'-dicyclohexyl-

carbodiimide. It is also possible to form amino or amido esters of the acids used as acylating agents in the presence of the starting material of formula II that is to be acylated, by reacting a mixture of the corresponding acid and amino starting materials in the presence of an N,N'-disubstituted carbodiimide, for example N,N'-dicyclohexylcarbodiimide, and of an N-hydroxyamine or N-hydroxyamide, for example N-hydroxysuccinimide, N-hydroxy-norbornane-2,3-dicarboximide or N-hydroxybenzotriazole, where appropriate in the presence of a suitable base, for example 4-dimethylaminopyridine or tetramethylguanidine.

In order to introduce a radical R_1 that is other than acyl (formyl), Process a) is preferably carried out by first reacting the starting material of formula II in a suitable solvent, such as dimethylformamide or tetrahydrofuran, with a suitable base, such as sodium bis(trimethylsilyl)amide in tetrahydrofuran or sodium hydride, at a temperature of preferably from -20°C to +70°C, especially from 0°C to room temperature, and then adding the compound of formula III, for example in a suitable solvent, such as tetrahydrofuran.

In order to introduce an acyl radical (formyl radical) R_1, Process a) is preferably carried out by reacting the starting material of formula II in a suitable solvent, such as methylene chloride, in the presence of a suitable base, such as triethylamine, with a reactive acid derivative of formula III, which may also be formed *in situ* from the corresponding acid, at a temperature of from 0°C to +150°C, for example under reflux. Alternatively, the starting material of formula II can first be reacted in a suitable solvent, such as absolute tetrahydrofuran, with a suitable base, such as sodium bis(trimethylsilyl)amide in tetrahydrofuran, at a temperature of from 0°C to room temperature, and then a reactive acid derivative of formula III can be added.

Process b):
The functional groups to be protected in the reactants of formulae IV and V and the protecting groups used for that purpose correspond to those mentioned in Process a). Functional groups that are intended to participate in the desired reaction, such as the group $-NH-CH_3$, are not, of course, protected. The introduction and removal of the protecting groups is also carried out analogously to the manner described in Process a). The radical X in a compound of formula V corresponds to the radical Y in the compound of formula III and the reagents of formula V are analogous to the reagents of formula III.

In order to introduce a radical R_2 that is other than acyl, Process b) is preferably carried

out by reacting the starting material of formula IV in a suitable solvent, such as dimethylformamide or a halogenated hydrocarbon, such as chloroform, in the presence of a suitable base, such as N,N-diisopropylethylamine, at a suitable temperature, such as room temperature or elevated temperature up to about +150°C, with a compound of formula V, the reaction being carried out at elevated temperature, for example under pressure in a closed vessel, such as a bomb tube, especially when X is an additional single bond the other end of which replaces a hydrogen atom in the radical R_2, for example when the compound of formula V is an oxirane or acrylonitrile. The reaction with oxiranes is preferably carried out in a lower alkanol, such as ethanol, as solvent.

In order to convert a compound of formula I obtained by Process a) or b) into a different compound of formula I, for example an ester grouping can be hydrolysed to carboxy or a carbonyl group can be reduced. The said hydrolysis is carried out, for example, in a manner known *per se* with dilute, for example 2-normal, sodium hydroxide solution in a lower alkanol, such as ethanol, at room temperature, and can also be seen as the removal of a protecting group. For the reduction of a carbonyl group, including a carbonyl group forming part of an amide or lactam group, reducing agents that come into consideration are, for example, complex metal hydrides, such as alkali metal aluminium hydrides and, especially, alkali metal borohydrides, for example lithium aluminium hydride, potassium borohydride, lithium borohydride and, especially, sodium borohydride, and derivatives thereof wherein one or more hydrogen atoms have been replaced by alkoxy radicals or by cyano, for example methoxysodium borohydride, tri-(tert-butoxy)lithium borohydride or di-(2-methoxyethoxy)-disodium lithium hydride or sodium cyanoborohydride, and also diborane.

Salt-forming groups in compounds of formulae II to V and salts thereof are those mentioned above for the compounds of formula I.

The salt formation, which is to be carried out if desired, or the freeing of the fundamental forms from their salts is carried out in a conventional manner that is generally known *per se*. Thus, compounds carrying carboxy groups are converted into corresponding salts with bases, especially into alkali metal salts, by treatment with a corresponding base, especially a compound giving an alkaline reaction, such as a hydroxide, carbonate or bicarbonate. The salts can be converted into free carboxy compounds by acidifying, for example with inorganic acids, such as especially hydrohalic acids. End products giving a basic reaction, for example amines, can be converted into their salts with acids, for

example by treatment with an acid suitable for salt formation, such as one of those mentioned above; conversely, by treating with agents that give a basic reaction, such as with inorganic hydroxides, carbonates and bicarbonates, or organic bases and ion-exchangers, such a basic fundamental form of an amine is freed.

Salts, such as the picrates, can also be used for the purification of the compounds obtained, by converting the free compounds into salts, separating these and recovering the free compounds from the salts again.

In view of the close relationship between the compounds in free form and in the form of their salts, hereinbefore and hereinafter any reference to the free compounds is to be understood as including also the corresponding salts (including quaternary salts) where appropriate and expedient.

The starting materials corresponding to formula IV wherein R_1 is hydrogen are known or can be prepared by processes that are known *per se*. The starting material corresponding to formula IV wherein R_1 and R_3 are each hydrogen, i.e. staurosporin, is commercially available and can be obtained by fermentation with the strain Streptomyces staurosporeus. That strain was deposited under number FERM P-3725 at the Fermentation Research Institute, Japan, in connection with Japanese Examined Patent Publication [Kokoku] No. 57-53076 which was published on 11.11.1982, see S. Omura *et al.*, J. Antibiot. 30, 275-281 (1977). Staurosporin derivatives corresponding to formula IV wherein R_3 is other than hydrogen are, for example, described by I. Takahashi *et al.*, J. Pharmacol. Exp. Ther. 255(3) (1990) 1218-1221 and in WO-A-8907-105-A (Applicant: Kyowa Hakko Kogyo KK, Japanese Priority No. 024571 of 4. 2. 1988). Compounds of formula II wherein R_3 is oxo are obtained, for example, from the corresponding compounds of formula II wherein R_3 is hydrogen by oxidation with chromium trioxide in pyridine. From the 7-oxo compounds so obtained the corresponding 7-hydroxy compounds wherein R_3 is hydroxy are obtained by reduction with sodium borohydride. Compounds corresponding to formula I wherein R_3 is hydroxy or oxo are also obtained as a by-product in the synthesis of compounds of formula I wherein R_3 is hydrogen. From the known staurosporin derivatives the starting materials of formulae II and IV that are still novel are obtained by appropriately carrying out reactions that are analogous to Process variants a) and b) described above.

The starting materials of formula V wherein R_2 is 2-(tetrahydropyran-4-yl-oxy)-lower

alkanoyl are obtained, for example, by reacting tetrahydropyran-4-ol with a corresponding chloro-lower alkanoic acid. In this procedure, tetrahydropyran-4-ol is first reacted in a suitable inert aprotic solvent, such as an acyclic or cyclic ether, such as dioxane, with a suitable base, such as sodium hydride. The suspension so obtained is added dropwise to a solution of a chloro-lower alkanoic acid in a suitable inert aprotic solvent, such as an acyclic or cyclic ether, such as dioxane. The reaction is carried out at from 0°C to 150°C, preferably from 20°C to 100°C, for example at the reflux temperature of the solvent used.

The compounds of formula I carrying a 2-(tetrahydropyran-4-yl-oxy)-lower alkanoyl radical are many times, for example more than 10 times, more soluble in water and other solvents than are other N-acyl-staurosporin derivatives, such as N-benzoylstaurosporin.

Unless stated otherwise, all of the processes described above, including the processes for removing protecting groups and the additional process measures, are carried out in a manner known *per se*, for example in the presence or absence of preferably inert solvents and diluents, if necessary in the presence of condensation agents or catalysts, at reduced or elevated temperature, for example in a temperature range of from approximately -70°C to approximately +150°C, especially from approximately -20°C to approximately +100°C, mainly from approximately 0°C to approximately +70°C, preferably from approximately 0°C to approximately +50°C, mainly at room temperature, in a suitable vessel and, if necessary, under an inert gas atmosphere, for example a nitrogen atmosphere.

In those processes, taking into consideration all of the substituents in the molecule, if necessary, for example if readily hydrolysable radicals are present, especially mild reaction conditions are to be used, such as short reaction times, the use of mild acidic or basic agents in low concentration, stoichiometric quantity ratios, selection of suitable catalysts, solvents and temperature and/or pressure conditions.

The invention relates also to those forms of the process in which a compound obtainable as intermediate at any stage of the process is used as starting material and the remaining steps are carried out, or the process is discontinued at any stage or a starting material is formed under the reaction conditions or is used in the form of a reactive derivative or salt. The starting materials used are preferably those which result in accordance with the process in the compounds described above as being especially valuable.

The present invention relates also to novel starting materials and/or intermediates and to

processes for the preparation thereof. The starting materials used and the reaction conditions chosen are preferably such that the compounds mentioned in this Application as being especially preferred are obtained.

The invention relates also to the use of the compounds of formula I and their pharmaceutically acceptable acid addition salts, preferably in the form of pharmaceutical compositions, for the therapeutic treatment of the human or animal body, especially in the case of the diseases mentioned above. The invention relates also to a method of removing existing multidrug resistance and of preventing the development of multidrug resistance in a warm-blooded animal in need of such treatment, wherein an effective dose that removes the multi-drug resistance and avoids the development thereof of a compound of formula I, or of a pharmaceutically acceptable salt thereof, is administered enterally, for example orally, or parenterally, for example intraperitoneally or intravenously, to that warm-blooded animal. The dose of the active ingredient depends *inter alia* upon the nature of the disease, the species to be treated and its size, the organism's state of defence and the mode of administration. For example, a daily dose of from 10 mg to 1000 mg, mainly from 50 mg to 500 mg, preferably from 70 mg to 300 mg, for example 150 mg, of a compound of formula I will be administered enterally or parenterally, for example intraperitoneally, to a warm-blooded animal of approximately 70 kg body weight. This total daily dose may be divided into 2 or 3 doses per day.

The invention relates also to pharmaceutical compositions that comprise an effective amount, especially an amount effective for the prophylaxis or treatment of one of the diseases mentioned above, of the active ingredient together with pharmaceutically acceptable carriers that are suitable for topical, enteral, for example oral or rectal, or parenteral, for example intraperitoneal, administration, and may be inorganic or organic and solid or liquid. For oral administration there are used especially tablets or gelatin capsules that comprise the active ingredient together with diluents, for example lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycerol, and/or lubricants, for example silica, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol. Tablets may also comprise binders, for example magnesium aluminium silicate, starches, such as corn, wheat or rice starch, gelatin, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, and, if desired, disintegrators, for example starches, agar, alginic acid or a salt thereof, such as sodium alginate, and/or effervescent mixtures, or adsorbents, colourings, flavourings and sweeteners. It is also possible to use the pharmacologically active compounds of the present invention in the

form of parenterally administrable compositions or infusion solutions. Such solutions are preferably isotonic aqueous solutions or suspensions, it being possible, for example in the case of lyophilised compositions that comprise the active ingredient on its own or together with a carrier, for example mannitol, for such solutions or suspensions to be made up prior to use. The pharmaceutical compositions may be sterilised and/or may comprise excipients, for example preservatives, stabilisers, wetting agents and/or emulsifiers, solubilisers, salts for regulating the osmotic pressure and/or buffers. The pharmaceutical compositions in question, which may, if desired, comprise other pharmacologically active substances, such as antibiotics, are prepared in a manner known *per se*, for example by means of conventional mixing, granulating, confectioning, dissolving or lyophilising processes, and comprise approximately from 0.01 % to 90 %, and in the case of lyophilised compositions up to 100 %, especially from approximately 0.1 % to approximately 50 %, most especially from 1 % to 30 %, active ingredient(s), an active ingredient concentration below 1 % being especially suitable for compositions for topical administration.

The following Examples illustrate the invention without limiting it in any way. The R_f values are determined on silica gel thin-layer plates (produced by Merck, Darmstadt, Germany). The ratio of the eluants to one another in the eluant mixtures used is given in parts by volume (v/v), and temperatures are given in degrees Celsius. In the case of optical rotation, the concentration, c, of the substance in the solvent or solvent mixture is given as a percentage (weight/volume).

Within the scope of this text, the following nomenclature is used to specify the compounds of formula I: the nitrogen atom $\underline{N}$-R_2 in the tetrahydropyran ring in formula I is designated "N". For example, N-BOC-staurosporin is a staurosporin derivative in which the radical R_2 is BOC. The nitrogen atom $\underline{N}$-R_1, on the other hand, is designated "6", as will be apparent from the numbering given in formula I. For example, 6-methyl-staurosporin is a staurosporin derivative in which the radical R_1 is methyl.

Abbreviations:
BOC: tertiary butoxycarbonyl
DMF: dimethylformamide
HPLC: high-pressure liquid chromatography
THF: tetrahydrofuran

Example 1: 2.2 ml of a 1-molar solution of sodium bis(trimethylsilyl)amide in tetrahydrofuran are added at room temperature under a nitrogen atmosphere to a solution of 1.13 g (0.002 mol) of N-BOC-staurosporin (described in Example 36 of EP-A-296110) in 10 ml of dry dimethylformamide and stirring is carried out for 1 hour. A solution of 0.14 ml (0.0022 mol) of methyl iodide in 2 ml of dimethylformamide is then added dropwise and stirring is continued at room temperature for 2 hours. The reaction mixture is poured onto ice and extracted with ethyl acetate. The organic phase is washed with cold 0.1-normal hydrochloric acid, dried over sodium sulfate and concentrated by evaporation. The residue is separated by means of flash-chromatography on silica gel (type 60, Merck, Darmstadt, Germany). Ethyl acetate/petroleum ether (1:1) are used as eluant. Two compounds that are separate from each other are obtained from the individual fractions, namely N-BOC-6-methyl-7-oxo-staurosporin, m.p. 180-185°C, $R_f = 0.58$ (methylene chloride:ethanol = 95:5), and N-BOC-6-methyl-staurosporin, m.p. 225-228°C, $R_f = 0.45$ (methylene chloride:ethanol = 95:5).

Example 2: 0.12 ml (0.0012 mol) of bromoacetic acid methyl ester is added at room temperature to 0.48 g (0.001 mol) of 6-methyl-staurosporin and 0.2 ml (0.00116 mol) of N,N-diisopropylethylamine in 8 ml of dimethylformamide and stirring is carried out at room temperature for 16 hours. The solution is concentrated by evaporation and the residue is purified by means of flash-chromatography (silica gel 60, methylene chloride:ethanol = 98:2). N-methoxycarbonylmethyl-6-methyl-staurosporin is obtained; m.p.145-150°C, $R_f = 0.43$ (methylene chloride:ethanol = 95:5).

The starting material is obtained as follows:

Step 2.1: 0.7 g (0.0012 mol) of N-BOC-6-methyl-staurosporin (see Example 1) is dissolved in 4 ml of ethyl acetate and, at room temperature, 4 ml of an ethyl acetate solution saturated with hydrochloric acid are added thereto. After 3.5 hours, the suspension is partitioned between ethyl acetate and sodium hydrogen carbonate solution, and the organic phase is separated, dried over sodium sulfate and concentrated by evaporation. Flash-chromatography on silica gel 60 using ethyl acetate/ethanol (8:2) yields 6-methyl-staurosporin, m.p. 210-215°C, $R_f = 0.28$ (ethyl acetate:ethanol = 8:2).

Example 3: 270 mg (0.49 mmol) of N-methoxycarbonylmethyl-6-methyl-staurosporin (see Example 2) are dissolved in 15 ml of methanol and, at room temperature, 0.3 ml (0.6 mmol) of 2-normal sodium hydroxide solution is added thereto. The reaction mixture

is then heated under reflux for 14 hours, is cooled, neutralised (pH 4-5) with 0.15 ml of glacial acetic acid and diluted with 10 ml of water. The precipitate is filtered off, washed with water and dried under a high vacuum. N-carboxymethyl-6-methyl-staurosporin is obtained; m.p. 215-218°C, R_f = 0.23 (methylene chloride:methanol:glacial acetic acid = 50:50:1).

Example 4: 1.65 ml of a 1-molar solution of sodium bis(trimethylsilyl)amide in tetrahydrofuran are added at room temperature under a nitrogen atmosphere to a solution of 0.85 g (0.0015 mol) of N-BOC-staurosporin in 8 ml of dry dimethylformamide and stirring is carried out for one hour. 0.2 ml (0.00165 mol) and, after 3 hours, a further 0.02 ml (0.000165 mol) of benzyl bromide are added thereto and stirring is carried out at room temperature for 2 hours. The reaction mixture is poured onto ice and extracted with ethyl acetate. The organic phase is washed with cold 0.1-normal hydrochloric acid, dried over sodium sulfate and concentrated by evaporation. The residue is separated by means of flash-chromatography on silica gel 60, using methylene chloride/ethanol (98:2) as eluant. N-BOC-6-benzyl-staurosporin is obtained, m.p. 205-207°C, R_f = 0.44 (methylene chloride/ethanol = 98:2), together with mixed fractions that yield, after chromatography once more in the same system, N-BOC-6-benzyl-7-oxo-staurosporin, m.p. 115-120°C, R_f = 0.59 (methylene chloride:ethanol = 98:2).

Example 5: 120 mg (0.21 mmol) of 6-benzyl-staurosporin and 31 mg (0.24 mmol) of N,N-diisopropylethylamine are introduced into 5 ml of dry dimethylformamide and, at room temperature, 28 mg (0.25 mmol) of chloroformic acid ethyl ester are added thereto. After 1 hour at room temperature, the reaction mixture is concentrated by evaporation under a high vacuum and the residue is purified by means of flash-chromatography using methylene chloride/ethanol (98:2) as eluant, to yield N-ethoxycarbonyl-6-benzyl-staurosporin; melting range 190 - 200°C, R_f = 0.28 (methylene chloride:ethanol = 98:2).

The starting compound is prepared as follows:

Step 5.1: Analogously to Step 2.1, there is obtained from 0.55 g (0.84 mmol) of N-BOC-6-benzyl-staurosporin 6-benzyl-staurosporin; m.p. 187-190°C, R_f = 0.34 (methylene chloride:ethanol = 95:5).

Example 6: 42.5 mg (0.283 mmol) of 1-hydroxybenzotriazole and 54.3 mg (0.283 mmol) of N-ethyl-N'-(3-diaminopropyl)carbodiimide hydrochloride (EDC) are added at 0°C to a

solution of 35.0 mg (0.218 mmol) of 2-(tetrahydropyran-4-yloxy)-acetic acid in 2 ml of absolute N,N-dimethylformamide, and stirring is carried out at 0°C under argon for 3 hours. There are then added to the colourless solution 84 mg (0.174 mmol) of crude 6-methyl-staurosporin (see Example 2) and stirring is carried out for 2 hours at 0° and for 18 hours at room temperature. The yellow solution so obtained is then concentrated to dryness by evaporation at 40° under a high vacuum. 3 ml of water are added to the residue and stirring is carried out at room temperature for 1/4 hour. The crystals are filtered off with suction and washed with water. The resulting crude product (yellow crystals) is further purified by flash-chromatography at 0.3 bar on 15 g of silica gel (type Si60, Merck 9385; 0.040-0.063 mm) in methylene chloride/ethanol (95:5) (10 ml fractions). Fractions 7-11 are combined and concentrated by evaporation at 30° under a high vacuum. 70 mg of yellowish crystals are obtained which are subjected to a further flash-chromatography at 0.3 bar on 15 g of silica gel (type Si60, Merck 9385; 0.040-0.063 mm) in methylene chloride/ethanol (98:2) (10 ml fractions). Fractions 7-12 are combined and concentrated by evaporation at 30° under a high vacuum. After a third flash-chromatography of the slightly contaminated product so obtained (yellowish crystals) at 0.3 bar on 15 g of silica gel (type Si60, Merck 9385; 0.040-0.063 mm) in methylene chloride/ethanol (95:5; 5 ml fractions), fractions 4-7 are combined and again concentrated by evaporation. Crystallisation of the residue (yellowish crystals) from 3 ml of ethyl acetate/cyclohexane (1:2) yields N-[2-(tetrahydropyran-4-yloxy)-acetyl]-6-methyl-staurosporin in the form of beige crystals of m.p. 183.4-185.8°C (sintering from 177°), that still contain 0.33 mol (1.0 %) of water; $[\alpha]_D^{20}$ = +155.9 +/- 2.3° (c = 0.440; chloroform).

Example 7: With stirring at room temperature under argon, 30 mg (0.75 mmol) of sodium hydride (approx. 60 %, in oil; Fluka, pract.) are added to a solution of 305 mg (0.5 mmol) of N-[2-(tetrahydropyran-4-yloxy)-acetyl]-staurosporin in 15 ml of absolute tetrahydrofuran, and the grey suspension so obtained is stirred at room temperature under argon for 3 hours. 128 mg (90 µl, d = 1.437; 0.75 mmol) of benzyl chloride (Fluka, purum) are then added thereto and the suspension, which is then yellow in colour, is stirred at room temperature for 20 hours. To the yellow solution now obtained 5 ml of water are added and the batch is diluted with 80 ml of methylene chloride. The organic phase is washed once with 0.1-normal hydrochloric acid, once with 30 ml of saturated sodium hydrogen carbonate solution and twice with 30 ml of water each time. The aqueous phases are then extracted once more with 50 ml of methylene chloride. All the methylene chloride phases are combined, dried over magnesium sulfate, filtered and concentrated by evaporation at 30°C under a high vacuum. The residue (yellow crystals) is dissolved in 30 ml of ethyl

acetate, and 30 ml of diethyl ether are added to this solution. The substance which precipitates is filtered off with suction, washed with diethyl ether and recrystallised from 5 ml of ethyl alcohol. The crude product (yellow crystals) is purified further by flash-chromatography at 0.4 bar on 100 g of silica gel (type Si60, Merck 9385; 0.040-0.063 mm) in ethyl acetate/petroleum ether (9:1, 25 ml fractions). Fractions 61-76 and 77-92 are combined and concentrated to dryness by evaporation at 30°C under a high vacuum. Beige crystals (I) are obtained from fractions 61-76 and yellow crystals (II) from fractions 77-92. II is flash-chromatographed once more (100 g of silica gel Si60, Merck 9385; 0.040-0.063 mm, eluant ethyl acetate/petroleum ether [9:1; 25 ml fractions]). Fractions 35-47 and 48-70 are combined and concentrated by evaporation again. Beige crystals (III) are obtained from fractions 35-47 and yellow crystals (IV) from fractions 48-70. I and III are combined and further purified once more by flash-chromatography on 150 g of silica gel (type Si60, Merck 9385; 0.040-0.063 mm) in ethyl acetate/-petroleum ether (9:1) (20 ml fractions). Fractions 86-120 are combined and concentrated by evaporation. The residue (beige crystals) crystallises from 22 ml of ethyl acetate/-diethyl ether (1:10). N-[2-(tetrahydropyran-4-yloxy)-acetyl]-6-benzyl-staurosporin is obtained in the form of beige crystals of m.p. 177-179° that still contain 0.4 mol (1.03 %) of water; $[\alpha]_D^{20}$= +106.2 +/- 2.0° (c = 0.509; chloroform/methanol= 1:1), R_f = 0.13 (ethyl acetate: petroleum ether = 95:5), R_f = 0.55 (methylene chloride:ethyl alcohol = 95:5), R_f = 0.61 (acetone).

Example 8: Analogously to Example 7, there is obtained from 315 mg (0.5 mmol) of N-[O-(tetrahydropyran-4-yl)-D-lactoyl]-staurosporin, 30 mg (0.75 mmol) of sodium hydride (approx. 60 % in oil; Fluka, pract.) and 128 mg (90 µl, d = 1.437; 0.75 mmol) of benzyl chloride, in the same reaction time and with repeated analogous flash-chromatography N-[O-(tetrahydropyran-4-yl)-D-lactoyl]-6-benzyl-staurosporin in the form of colourless crystals of m.p. 268-270°C (sintering from 262°, from ethyl acetate) that still contain 0.23 mol (0.57 %) of water; $[\alpha]_D^{20}$ = +112.5 +/- 2.2° (c = 0.446; chloroform/-methanol= 1:1), R_f = 0.21 (ethyl acetate:petroleum ether= 95:5), R_f = 0.5 (methylene chloride:ethyl alcohol = 95:5), R_f = 0.74 (acetone).

Example 9: Human KB-31 (sensitive) and KB-8511 (drug-resistant, P-glycoprotein [Pgp] overexpressing) cells are incubated under a 5 % carbon dioxide atmosphere in MEM-Alpha-Medium, with the addition of ribonucleosides and deoxyribonucleosides and in the presence of 5 % foetal calf serum, 50 units/ml of the antibiotic penicillin and 50 µg/ml of the antibiotic streptomycin. The KB-8511 cells are kept as stock in the presence of

10 ng/ml of the antineoplastically active substance Colcemid (demecolcine). To determine the inhibition of the cell growth, batches of 1500 cells (without the addition of Colcemid) are sown in 96-well microtitre plates and incubated overnight under the conditions mentioned above. The test substance (A: the antineoplastically active substance vinblastine, B: the compound of formula I N-BOC-6-methyl-staurosporin) is added in serial dilutions on day 1. The plates are then incubated under the conditions mentioned above for 4 days. During that time, the control cells undergo several cell divisions. After incubation, the cells are fixed with 3.3 % (w/v) aqueous glutaraldehyde solution, washed with water and stained with 0.05 % (w/v) methylene blue solution. After washing, the dye is eluted with 3 % (w/v) aqueous hydrochloric acid. The optical density (OD) per well, which is directly proportional to the number of cells, is then measured with a photometer at 665 nm. The IC_{50} values are calculated by means of a computer system, using the formula

$$[OD_{665}(test)-OD_{665}(start)]/[OD_{665}(control)-OD_{665}(start)] \times 100$$

The IC_{50} values are defined as being those concentrations of active ingredient at which the number of cells per well at the end of the incubation period amounts to only 50 % of the number of cells in the control cultures.

test substance [concentration]	% growth of KB 8511 cells:
A [50 ng/ml]	104
A [25 ng/ml]	104
A [12.5 ng/ml]	108
A [6.25 ng/ml]	106
A [3.13 ng/ml]	95
B [1 µmol]	95
B [1 µmol] + A [50 ng/ml]	0
B [1 µmol] + A [25 ng/ml]	0
B [1 µmol] + A [12.5 ng/ml]	0
B [1 µmol] + A [6.25 ng/ml]	0
B [1 µmol] + A [3.13 ng/ml]	0

test substance A: vinblastine

test substance B: N-BOC-6-methyl-staurosporin

Example 10: The following results are obtained analogously to Example 9, using test substance C (= N-ethoxycarbonyl-6-benzyl-staurosporin) instead of test substance B:

test substance [concentration]	% growth of KB 8511 cells:
A [400 ng/ml]	0
A [200 ng/ml]	31
A [100 ng/ml]	83
A [50 ng/ml]	93
A [25 ng/ml]	93
A [12.5 ng/ml]	96
A [6.25 ng/ml]	101
A [3.13 ng/ml]	99
C [1 µmol]	92
C [1 µmol] + A [400 ng/ml]	0
C [1 µmol] + A [200 ng/ml]	0
C [1 µmol] + A [100 ng/ml]	0
C [1 µmol] + A [50 ng/ml]	0
C [1 µmol] + A [25 ng/ml]	0
C [1 µmol] + A [12.5 ng/ml]	0
C [1 µmol] + A [6.25 ng/ml]	0
C [1 µmol] + A [3.13 ng/ml]	0
C [0.1 µmol]	92
C [0.1 µmol] + A [400 ng/ml]	0
C [0.1 µmol] + A [200 ng/ml]	0
C [0.1 µmol] + A [100 ng/ml]	0
C [0.1 µmol] + A [50 ng/ml]	10
C [0.1 µmol] + A [25 ng/ml]	24
C [0.1 µmol] + A [12.5 ng/ml]	53
C [0.1 µmol] + A [6.25 ng/ml]	73
C [0.1 µmol] + A [3.13 ng/ml]	82

test substance A: vinblastine

test substance C: N-ethoxycarbonyl-6-benzyl-staurosporin

Example 11: The following results are obtained analogously to Example 9, using test substance D (= N-[2-(tetrahydropyran-4-yloxy)-acetyl]-6-methyl-staurosporin) instead of test substance B:

test substance [concentration]	% growth of KB 8511 cells:
A [400 ng/ml]	0
A [200 ng/ml]	25
A [100 ng/ml]	73
A [50 ng/ml]	86
A [25 ng/ml]	89
A [12.5 ng/ml]	100
A [6.25 ng/ml]	92
A [3.13 ng/ml]	95
C [1 µmol]	86
C [1 µmol] + A [400 ng/ml]	0
C [1 µmol] + A [200 ng/ml]	0
C [1 µmol] + A [100 ng/ml]	0
C [1 µmol] + A [50 ng/ml]	0
C [1 µmol] + A [25 ng/ml]	0
C [1 µmol] + A [12.5 ng/ml]	0
C [1 µmol] + A [6.25 ng/ml]	0
C [1 µmol] + A [3.13 ng/ml]	0
C [0.1 µmol]	87
C [0.1 µmol] + A [400 ng/ml]	0
C [0.1 µmol] + A [200 ng/ml]	2
C [0.1 µmol] + A [100 ng/ml]	49
C [0.1 µmol] + A [50 ng/ml]	74
C [0.1 µmol] + A [25 ng/ml]	87
C [0.1 µmol] + A [12.5 ng/ml]	96
C [0.1 µmol] + A [6.25 ng/ml]	87
C [0.1 µmol] + A [3.13 ng/ml]	100

test substance A: vinblastine

test substance D: N-[2-(tetrahydropyran-4-yloxy)-acetyl]-6-methyl-staurosporin

Example 12: The following results are obtained analogously to Example 9, using test substance E (= N-BOC-6-benzyl-staurosporin) instead of test substance B:

test substance [concentration]	% growth of KB 8511 cells:
A [400 ng/ml]	0
A [200 ng/ml]	25
A [100 ng/ml]	73
A [50 ng/ml]	86
A [25 ng/ml]	89
A [12.5 ng/ml]	100
A [6.25 ng/ml]	92
A [3.13 ng/ml]	95
C [1 µmol]	91
C [1 µmol] + A [400 ng/ml]	0
C [1 µmol] + A [200 ng/ml]	0
C [1 µmol] + A [100 ng/ml]	0
C [1 µmol] + A [50 ng/ml]	0
C [1 µmol] + A [25 ng/ml]	0
C [1 µmol] + A [12.5 ng/ml]	1
C [1 µmol] + A [6.25 ng/ml]	9
C [1 µmol] + A [3.13 ng/ml]	35
C [0.1 µmol]	93
C [0.1 µmol] + A [400 ng/ml]	0
C [0.1 µmol] + A [200 ng/ml]	20
C [0.1 µmol] + A [100 ng/ml]	64
C [0.1 µmol] + A [50 ng/ml]	86
C [0.1 µmol] + A [25 ng/ml]	92
C [0.1 µmol] + A [12.5 ng/ml]	101
C [0.1 µmol] + A [6.25 ng/ml]	102
C [0.1 µmol] + A [3.13 ng/ml]	99

test substance A: vinblastine

test substance E: N-BOC-6-benzyl-staurosporin

Example 13: Tablets, each comprising 20 mg of active ingredient, for example one of the compounds of formula I described in the preceding Examples, are prepared in the usual manner with the following composition:

Composition	
active ingredient	20 mg
wheat starch	60 mg
lactose	50 mg
colloidal silicic acid	5 mg
talc	9 mg
magnesium stearate	1 mg
	145 mg

Preparation: The active ingredient is mixed with a portion of the wheat starch, with the lactose and the colloidal silicic acid, and the mixture is forced through a sieve. A further portion of the wheat starch is made into a paste with 5 times the amount of water on a water bath and the powder mixture is kneaded with that paste until a slightly plastic mass has been produced.

The plastic mass is pressed through a sieve of approximately 3 mm mesh size and dried, and the resulting dry granules are forced through a sieve once more. The remainder of the wheat starch, the talc and the magnesium stearate are then added and the mixture is compressed to form tablets each weighing 145 mg and having a breaking notch.

Example 14: Capsules, each comprising 25 mg of active ingredient, for example one of the compounds of formula I described in the preceding Examples, are prepared as follows:

Composition	
active ingredient	25.0 mg
gelucire 44/14	183.3 mg

(gelucire 44/14 is a mixture of esters of saturated C_8-C_{18}-fatty acids with glycerol and polyethylene glycol having a molecular weight of approximately 1500; produced by: Gatte-fossé, F-69800 Saint Priest, France).

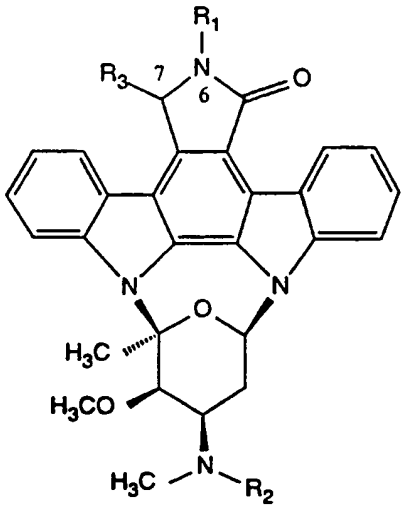
Preparation

A portion of the gelucire 44/14 is melted at a temperature of from 50°C to 100°C. The active ingredient is mixed with the liquid gelucire 44/14 in a heated mortar to form a paste. The remainder of the gelucire 44/14 is then also melted and is added to the paste. The mixture is stirred at 50°C until a solution is obtained. This is introduced into the capsules while warm and is cooled. The wax so obtained comprises 12 % by weight active ingredient.

The wax-like dispersion can also be processed in water by ultrasound treatment to form a milky liquid that can be administered orally.

What is claimed is:

1. A staurosporin derivative of formula I



(I),

wherein

R_1 is formyl, an aliphatic hydrocarbon radical having up to 29 carbon atoms that is unsubstituted or substituted by aryl, or is an aryl radical,

R_2 is an aliphatic, carbocyclic, carbocyclic-aliphatic, heterocyclic or heterocyclic-aliphatic radical each having up to 29 carbon atoms that is other than C_1-C_5alkyl, or is a heterocyclic or heterocyclic-aliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms, or is an acyl radical having up to 30 carbon atoms that is other than benzoyl, benzyloxycarbonyl, lower alkanoyl or α-aminoacyl having a free or protected amino group, and

R_3 is hydrogen, hydroxy, lower alkoxy or oxo,

or a salt of such a compound of formula I having at least one salt-forming group.

2. A derivative of formula I according to claim 1, wherein R_1 is formyl, an aliphatic hydrocarbon radical having up to 29 carbon atoms that is unsubstituted or substituted by aryl and that is other than unsubstituted lower alkyl, or is an aryl radical, or a salt of such a compound of formula I having at least one salt-forming group.

3. A derivative of formula I according to claim 1, wherein R_1 is lower alkyl or benzyl, R_2 is lower alkoxycarbonyl or tetrahydropyran-4-yloxy-lower alkanoyl, or is lower alkyl

substituted by lower alkoxycarbonyl or by carboxy, and R_3 is hydroxy, lower alkoxy, hydrogen or oxo, or a salt of such a compound having at least one salt-forming group.

4. A derivative of formula I according to claim 1, wherein R_1 is lower alkyl or benzyl, R_2 is tetrahydropyran-4-yloxy-lower alkanoyl, or is lower alkyl substituted by lower alkoxycarbonyl or by carboxy, and R_3 is hydroxy, lower alkoxy, hydrogen or oxo, or a salt of such a compound having at least one salt-forming group.

5. A derivative of formula I according to claim 1, wherein R_1 is benzyl, R_2 is lower alkoxycarbonyl or tetrahydropyran-4-yloxy-lower alkanoyl, or is lower alkyl substituted by lower alkoxycarbonyl or by carboxy, and R_3 is hydroxy, lower alkoxy, hydrogen or oxo, or a salt of such a compound having at least one salt-forming group.

6. A derivative of formula I according to claim 1, wherein R_1 is methyl or benzyl, R_2 is ethoxycarbonyl, tert-butoxycarbonyl, 2-tetrahydropyran-4-yloxy-acetyl or (D)-O-tetrahydropyran-4-yl-lactoyl, or is lower alkyl substituted by methoxycarbonyl or by carboxy, and R_3 is hydrogen or oxo, or a salt of such a compound having at least one salt-forming group.

7. A derivative of formula I according to claim 1, wherein R_1 is methyl or benzyl, R_2 is 2-tetrahydropyran-4-yloxy-acetyl or (D)-O-tetrahydropyran-4-yl-lactoyl, or is lower alkyl substituted by methoxycarbonyl or by carboxy, and R_3 is hydrogen or oxo, or a salt of such a compound having at least one salt-forming group.

8. A derivative of formula I according to claim 1, wherein R_1 is benzyl, R_2 is ethoxycarbonyl, tert-butoxycarbonyl, 2-tetrahydropyran-4-yloxy-acetyl or (D)-O-tetrahydropyran-4-yl-lactoyl, or is lower alkyl substituted by methoxycarbonyl or by carboxy, and R_3 is hydrogen or oxo, or a salt of such a compound having at least one salt-forming group.

9. N-Ethoxycarbonyl-6-benzyl-staurosporin according to claim 1.

10. N-BOC-6-methyl-staurosporin according to claim 1.

11. A derivative of formula I according to claim 1, selected from
N-BOC-6-methyl-7-oxo-staurosporin,
N-methoxycarbonylmethyl-6-methyl-staurosporin,

N-carboxymethyl-6-methyl-staurosporin or a pharmaceutically acceptable salt thereof,
N-BOC-6-benzyl-staurosporin,
N-[2-(tetrahydropyran-4-yloxy)-acetyl]-6-methyl-staurosporin,
N-[2-(tetrahydropyran-4-yloxy)-acetyl]-6-benzyl-staurosporin and
N-[O-(tetrahydropyran-4-yl)-D-lactoyl]-6-benzyl-staurosporin.

12. A compound of formula I according to any one of claims 1 to 11 for use in a method for the therapeutic treatment of the human or animal body.

13. A pharmaceutical composition that comprises a compound of formula I according to any one of claims 1 to 11 together with a pharmaceutical carrier.

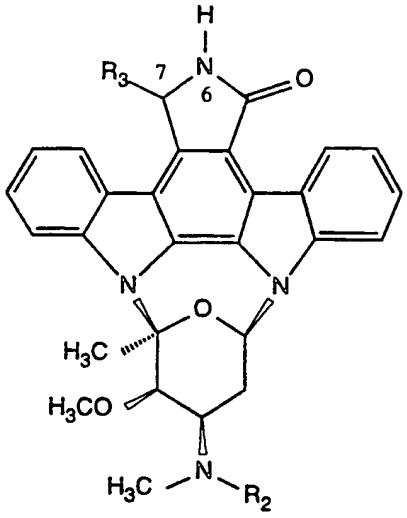
14. The use of a compound of formula I according to any one of claims 1 to 11 for avoiding or removing multidrug resistance to anti-tumour agents.

15. The use of a compound of formula I according to any one of claims 1 to 11 for the preparation of pharmaceutical compositions intended for use in avoiding or removing multidrug resistance to anti-tumour agents.

16. A method of avoiding or removing multidrug resistance to anti-tumour agents in a warm-blooded animal in need of such treatment, wherein a dose of a compound of formula I according to any one of claims 1 to 11 that is effective for avoiding or removing multidrug resistance to anti-tumour agents is administered to that warm-blooded animal.

17. A process for the preparation of a compound of formula I according to claim 1, which comprises

a) reacting a compound of formula II



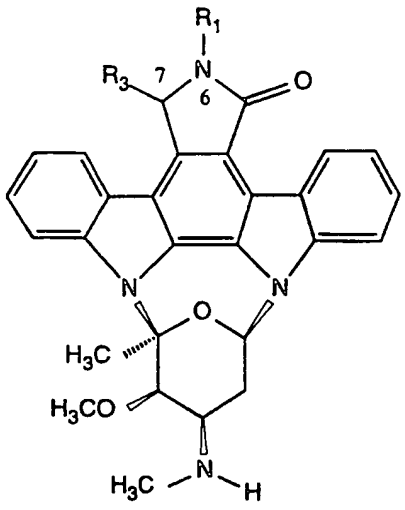
(II),

wherein the substituents are as defined above, any functional groups present therein being, if necessary, in protected form, or a salt of such a compound having at least one salt-forming group, with a compound of formula

R_1Y (III),

wherein R_1 is as defined above, any functional groups present therein being, if necessary, in protected form, and Y is a leaving group or an additional single bond the other end of which replaces a hydrogen atom in the radical R_1, or with a salt of such a compound having at least one salt-forming group, and removing any protecting groups, or

b) reacting a compound of formula IV



(IV),

wherein the substituents are as defined above, any functional groups present therein being, if necessary, in protected form, or a salt of such a compound having at least one salt-forming group, with a compound of formula

R_2X (V),

wherein R_2 is as defined above, any functional groups present in the radical R_2 being, if necessary, in protected form, and X is a leaving group or an additional single bond the other end of which replaces a hydrogen atom in the radical R_2, or with a salt of such a compound having at least one salt-forming group, and removing any protecting groups, and, if desired, converting a resulting compound of formula I into a different compound of formula I and/or converting a compound of formula I obtained in free form into a salt thereof and/or converting a compound of formula I obtained in the form of a salt into its free form or into a different salt.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/EP 95/01911

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D498/22 C07H19/044 A61K31/55 A61K31/70
//(C07D498/22,311:00,273:00,209:00,209:00,209:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP,A,0 630 898 (KYOWA) 28 December 1994 see claims 1,2	1,13
X	& WO,A,94 06799 31 March 1994	1,13
P,X	EP,A,0 643 966 (KYOWA) 22 March 1995 see claims 1,3,4	1,13,14
A	EP,A,0 383 919 (KYOWA) 29 August 1990 see page 1; claim 1	1,13
P,A	WO,A,95 00520 (CIBA-GEIGY) 5 January 1995 see page 4; claim 1	1,13,14

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 August 1995	- 9. 08. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Voyiazoglou, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In. .ational application No.
PCT/EP 95/01911

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 16 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ...nal Application No
PCT/EP 95/01911

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0630898	28-12-94	CA-A-	2123895	03-03-94
		WO-A-	9406799	31-03-94
WO-A-9406799	31-03-94	CA-A-	2123895	03-03-94
		EP-A-	0630898	28-12-94
EP-A-0643966	22-03-95	WO-A-	9420106	15-09-94
EP-A-0383919	29-08-90	WO-A-	8907105	10-08-89
WO-A-9500520	05-01-95	AU-B-	7000094	17-01-95

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